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CANADIAN FORESTRY SERVICE
TOXIC SUBSTANCES PROJECT
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ANNUAL REPORT

by

CANADIAN FORESTRY SERVICE

for

RESEARCH MANAGEMENT DIVISION
Alberta Environment

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PREFACE

In the past, a yearly report was submitted by the Canadian Forestry Service to the Research Management Division (formerly AOSERP) of Alberta Environment which attempted to write up in scientific form the work on air pollutants that had been carried out during the past year. Since much of the work had not been completed by March, or had not been synthesized adequately, detailed review of these incomplete and tentative findings was inappropriate and often lead to unnecessary criticism. In addition, since the bulk of research findings were published in the scientific literature and not included in annual reports, these reports did not accurately reflect what had actually been accomplished.

In order to try to provide a better product to the Research Management Division which will accurately demonstrate present research accomplishments as well as future goals, this report is organized in chronological order and includes all reports and articles that have been produced through the cooperation between Canadian Forestry Service and Alberta Environment in 1982. It is hoped that inclusion of published papers (the most appropriate reflection of scientific productivity) will provide the Research Management Division with a better appreciation of how their support has helped to promote scientific understanding in the field of pollutant responses of trees and forest systems in the Boreal Forest.

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INTRODUCTION

Industrial effluents discharged into the atmosphere in a number of locations have real, imagined, or potentially deleterious effects on forest vegetation and soils. Government agencies and the general public have expressed concern whereas industrial groups are apprehensive as to restrictions which may be applied to their operations. Since, in many cases, regulatory agencies lack essential scientific information describing cause and effect relationships, the Canadian Forestry Service has been involved in cooperative research programs, detection and assessment surveys and advisory services with provincial government agencies, industry and the public.

The objectives of the Toxic Substances Project are:

1. To determine the impact of pollutant individually as well as in mixtures on native soils and their ability to support vegetation.
2. To determine the effect of pollutants on the previsible (physiological and biochemical) and visible injury symptoms of boreal forest plant species.
3. Provide federal, provincial and industrial resource managers with techniques and methodology for detecting and assessing air pollution impact on forest vegetation and soils.

In the 1981 annual report, eight more specific objectives were identified for this project.

1. Determine the effect of pollutants in the soil solution on vascular plant functioning.
   Status: See section 4.3
2. Determine the influence of the soil litter on the availability and mobility of toxic elements.
   Status: Part of 4.3 and an integral part of an industry supported sister study in the Rocky Mountain House area.
3. Determine the influence of pollutants on soil biological processes relating to nutrient status.
   Status: Part of Rocky Mountain House study but deferred to 1983.
4. Determine field soil pollutant content; variability, horizontal and vertical distribution.
   Status: Sections 2.2.1, 5.2 and 5.3.
5. Determine the influence of SO_2 concentration and the duration of fumigation on jack pine physiology and growth.
   Status: See section 5.1.

6. Develop methods to determine previsual and transitory effects of pollutants on plant functioning.
   Status: See sections 2.1.1, 2.1.2, 2.1.3, 2.1.4, 2.1.5, 2.2.2, 3.1 and 4.2.
7. Determine the influence of SO_2 concentration and time of exposure on the activity of the epiphytic lichen *Evernia mesomorpha*.
   Status: See section 4.1.
8. Partition pollutant effects in jack pine into biophysical and biochemical components.
   Status: See section 5.1.

CANADIAN FORESTRY SERVICE

TOXIC SUBSTANCES PROJECT

1982

PUBLICATIONS

1. BIOCHEMISTRY AND PHYSIOLOGY
2. GENERAL AND ECOLOGICAL

BIOCHEMICAL AND PHYSIOLOGICAL PUBLICATIONS

5

RIBULOSE BIPHOSPHATE CARBOXYLASE AND GLYCOLLATE OXIDASE FROM JACK PINE: EFFECTS OF SULPHUR DIOXIDE FUMIGATIONS

by

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RIBULOSE BIPHOSPHATE CARBOXYLASE AND GLYCOLLATE OXIDASE FROM JACK PINE: EFFECTS OF SULPHUR DIOXIDE FUMIGATION

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Key Word Index—*Pinus banksiana*; Pinaceae; jack pine; ribulose bisphosphate carboxylase; glycollate oxidase; sulphur dioxide; SO_3^{2-}; SO_4^{2-}.

Abstract—Ribulose biphosphate (RuBP) carboxylase and glycollate oxidase were partially purified from jack pine (*Pinus banksiana* Lamb.) needles. Preincubation of RuBP carboxylase with HCO_3^- and Mg^{2+} markedly stimulated its activity. RuBP carboxylase showed hyperbolic reaction kinetics with respect to HCO_3^-, Mg^{2+}, and RuBP. Both SO_3^{2-} and SO_4^{2-} inhibited RuBP carboxylase, but SO_3^{2-} was more inhibitory than SO_4^{2-}. The SO_3^{2-} inhibition was competitive with respect to HCO_3^- (whether SO_3^{2-} was present during activation or was added to the activated enzyme), while the SO_4^{2-} inhibition was non-competitive with respect to HCO_3^-. Glycollate oxidase was inhibited more severely by low concentrations of SO_3^{2-} than by SO_4^{2-}. Fumigation of jack pine seedlings with 0.34 ppm sulphur dioxide for 24 and 48 hr produced a considerable decline in the activities of these enzymes, but 1 hr of fumigation produced no effect. During the longer exposures the sulphur content of the needles increased considerably, although the needles showed no visible injury. It is suggested that the accumulation of SO_3^{2-} and SO_4^{2-} in the needles following sulphur dioxide exposure influenced the enzyme activities.

INTRODUCTION

In leaves, photosynthetic carbon dioxide fixation is primarily carried out by ribulose bisphosphate (RuBP) carboxylase, an enzyme which is widely distributed in plant leaves. In C_3 plants, RuBP carboxylase can account for up to 60% of the soluble proteins in the leaves [1]. In *Pinus banksiana* Lamb. needles, photosynthetic carbon dioxide fixation occurs exclusively by the C_3-type mechanism; as in other C_3 species, RuBP carboxylase represents a major component of the total soluble proteins [Khan, A. A. and Malhotra, S. S., unpublished results]. In C_3 plants, carboxylation by RuBP carboxylase may be a rate-limiting reaction since changes in carbon dioxide concentration or enzyme activity affect photosynthesis [2–4]. This enzyme also acts as an oxygenase [5–7], catalysing cleavage of RuBP to phosphoglycollic and phosphoglyceric acids. Glycollic acid, derived from phosphoglycollic acid, is further metabolized by glycollate oxidase in the photorespiratory pathway. The activities of RuBP carboxylase and glycollate oxidase are, therefore, important for the photosynthetic process. In spite of the key roles of these enzymes in photosynthesis, their activities in conifers have received little attention. In this study we characterized partially purified RuBP carboxylase and glycollate oxidase from *P. banksiana*.

Sulphur dioxide, one of the major airborne pollutants, can petetrate plant foliage through stomata and can cause metabolic or physical injury. Inhibition of net photosynthesis in crop species [8–12] and forest vegetation [13] has often been observed after sulphur dioxide fumigation.

A reduction in photorespiration has been suggested as the reason for decreased synthesis of glycine and serine following exposure to sulphur dioxide [14]. Since these amino acids can also be synthesized by alternate pathways, a decrease in their biosynthesis cannot be linked exclusively to inhibition of photorespiration. A direct sulphur dioxide effect on reactions linked to photorespiration therefore remains to be demonstrated [15]. In view of the essential roles of RuBP carboxylase and glycollate oxidase in photosynthesis and photorespiration, respectively, we examined the effects of gaseous sulphur dioxide, SO_3^{2-} and SO_4^{2-} on their activities.

RESULTS AND DISCUSSION

Extraction, partial purification and characterization of RuBP carboxylase

RuBP carboxylase activity in the jack pine needle tissue was 50% higher in the mature, dark green needles than in the pale green, growing needles; therefore, mature needles were used for experimental purposes. Extraction of the enzyme was best achieved with a phosphate buffer (pH 7.5, 0.1 M) containing PVP-10 and magnesium chloride. The use of other buffers (Tris, Tes and borate) resulted in either no improvement or a partial loss of the enzyme activity. Similarly, use of PVP-40 or insoluble PVP resulted in preparations with a lower enzyme activity than PVP-10. Addition of dithioerythritol (DTE) and ascorbic acid did not improve the enzyme activity but prevented browning of the enzyme extract due to oxidation of tissue phenolics.

The partial purification of RuBP carboxylase from pine needles indicated that *ca* 95% of the total activity present in the initial homogenate was present in the 120000 *g* supernatant (Table 1). A major portion of this activity precipitated upon 60% saturation with ammonium sulphate. In order to assay enzyme activity at this stage, residual ammonium sulphate was removed by filtration on Biogel P-2. The enzyme extract was then further purified on a column of Sepharose 6B. The activity was eluted immediately after the void volume in a colourless fraction. Other protein-containing fractions were primarily brown and were devoid of enzyme activity. The overall recovery of the enzyme activity in the colourless fraction was 60–70%.

The enzyme was stable (85–90% retention of activity) at room temperature for at least 12 hr, but cold storage (0° or in frozen condition) resulted in a considerable decrease in its activity and the appearance of fine protein fibres in the solution. RuBP carboxylase from tobacco leaves has also been shown to undergo cold inactivation [16], and recently it was demonstrated that the enzyme from puma rye undergoes reversible cold inactivation, probably as a result of conformational alterations [17].

Pine RuBP carboxylase showed optimum activity at pH 7.5 in Tris buffer. The activity was stimulated considerably by preincubation with HCO_3^-, $MgCl_2$ or RuBP, but the stimulation was greatest when the enzyme was preincubated simultaneously with HCO_3^- and $MgCl_2$. Similar results have been reported with RuBP carboxylase from other plants [18–21]. A preincubation time of 2–5 min produced optimum activity.

The reaction kinetics of RuBP carboxylase with respect to RuBP, magnesium chloride and HCO_3^- were found to be hyperbolic in nature (Michaelis–Menten type). The apparent K_m values for RuBP and Mg^{2+} were 0.3 and 1.5 mM, respectively. Since the binding of HCO_3^-/CO_2 with the enzyme is known to influence the enzyme's conformation and affinity for HCO_3^-/CO_2 in preparations from other plants [22–24], the substrate kinetics for HCO_3^- with pine needle enzyme were studied under two sets of conditions. In one set, the enzyme was preincubated with varying amounts of HCO_3^-, and the reaction was started with RuBP. The apparent K_m in this system was 11.8 mM. In the other set, the enzyme was activated first by preincubating it with 10 mM HCO_3^- plus 20 mM magnesium chloride. The assay was then started using the enzyme and varying amounts of HCO_3^- (allowance was made for the residual amount of HCO_3^- in the enzyme extract). In this case, the apparent K_m for HCO_3^- was 3.6 mM. This indicates that initial binding of the enzyme with HCO_3^- markedly increased the enzyme's affinity for HCO_3^- during RUBP carboxylation and further shows that RUBP carboxylase from pine needles behaves similarly to that from spinach [22]. While this work was in progress, Gezelius and Hallgren [25] reported similar K_m values for HCO_3^- with unpurified extracts of *Pinus silvestris*.

Effects of SO_3^{2-} and SO_4^{2-} on RuBP carboxylase

The addition of SO_3^{2-} or SO_4^{2-} markedly inhibited RuBP carboxylase activity. SO_3^{2-} was more inhibitory than SO_4^{2-}: 5 mM SO_3^{2-} produced a 50% reduction in activity, while 10 mM or higher concentrations of SO_4^{2-} were required for a similar effect. This is in contrast to the unpurified enzyme from *P. silvestris* [25], for which both SO_3^{2-} and SO_4^{2-} were equally inhibitory.

To assess the specific nature of SO_3^{2-} and SO_4^{2-} inhibition on the enzyme, we used the enzyme preparations purified on Sepharose 6B. This was necessary in order to remove interference from SO_3^{2-} oxidizing enzyme systems. Furthermore, the assays were run under a nitrogen atmosphere. In experiments with SO_3^{2-}, the enzyme activity was measured under two sets of experimental conditions. In set 1, SO_3^{2-} was present during the activation step (preincubation), while in set 2 it was added to the activated enzyme. In both, the SO_3^{2-} effect was studied with respect to HCO_3^-. The results in Figs. 1 and 2 show that under both conditions SO_3^{2-} caused an inhibition that was competitive with respect to HCO_3^-. The values of apparent K_i for SO_3^{2-} were 2.2 mM for set 1 and 0.9 mM for set 2. A similar type of competitive inhibition has been reported for spinach chloroplast enzyme when SO_3^{2-} was added during the activation step [26]. The K_i for SO_3^{2-} for spinach enzyme was 3 mM, which is close to the value reported here (2.2 mM). Recently, however, Gezelius and Hallgren [25] reported that SO_3^{2-} inhibition was non-com-

Table 1. RuBP carboxylase partial purification from *P. banksiana* needles

Purification	Total activity (units)	Total protein (mg)	Specific activity (units/mg protein)	Overall recovery (%)
Initial homogenate (crude extract)	57	223	0.26	100.0
120000 *g* supernatant	54	87	0.62	95.0
$(NH_4)_2SO_4$ (0–60% fraction)	47	71	0.66	82.0
Sepharose 6B fraction	38	29	1.30	67.0

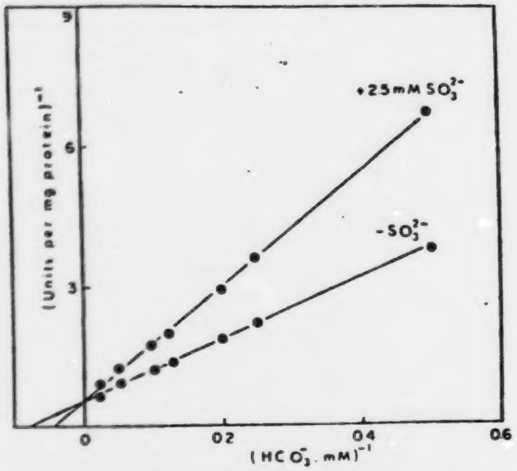

Fig. 1. Effect of SO_3^{2-} on RUBP carboxylase. A double reciprocal plot.

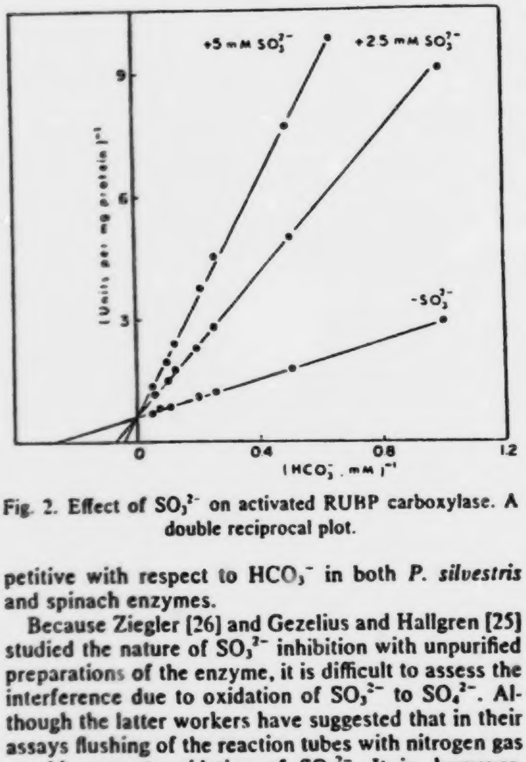

Fig. 2. Effect of SO_3^{2-} on activated RUBP carboxylase. A double reciprocal plot.

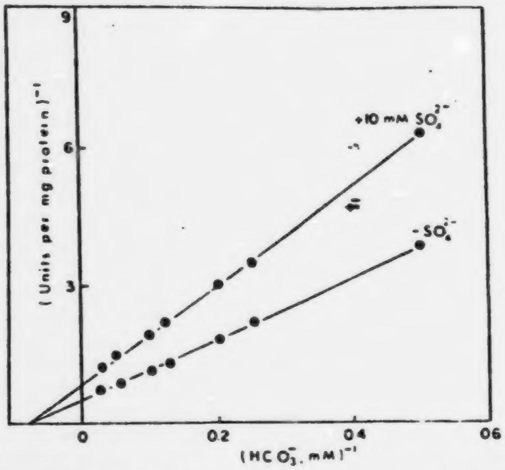

Fig. 3. Effect of SO_4^{2-} on RUBP carboxylase. A double reciprocal plot.

petitive with respect to HCO_3^- in both *P. silvestris* and spinach enzymes.

Because Ziegler [26] and Gezelius and Hallgren [25] studied the nature of SO_3^{2-} inhibition with unpurified preparations of the enzyme, it is difficult to assess the interference due to oxidation of SO_3^{2-} to SO_4^{2-}. Although the latter workers have suggested that in their assays flushing of the reaction tubes with nitrogen gas would prevent oxidation of SO_3^{2-}. It is, however, possible that their crude enzyme preparations may have contained an active SO_3^{2-} oxidizing system that could rapidly oxidize added SO_3^{2-} before complete anaerobic conditions were established. We have found that crude extracts and chloroplasts of *P. banksiana* needles contain very active SO_3^{2-} oxidizing systems [Khan, A. A. and Malhotra, S. S., unpublished results]. If crude extracts of the enzymes used by Gezelius and Hallgren [25] also contained active SO_3^{2-} oxidizing systems, it can be assumed that an appreciable amount of the added SO_3^{2-} was converted to SO_4^{2-} during flushing with nitrogen gas. In any case, with partially purified RuBP carboxylase of *P. banksiana* (which contained no SO_3^{2-} oxidizing activity) we were unable to obtain non-competitive inhibition by added SO_3^{2-}. Furthermore, we observed that our purified enzyme was inhibited by SO_4^{2-} and that the inhibition was non-competitive with respect to HCO_3^- (Fig. 3). Similar results have been reported for spinach enzyme [27]. The K_i for SO_4^{2-} was considerably higher (17.5 mM) than the K_i for SO_3^{2-} (2.2 mM). The similarity between the non-competitive inhibition of SO_4^{2-} that we found using a partially purified enzyme preparation and that of SO_3^{2-} in a crude extract of *P. silvestris* [25] suggests that in *P. silvestris* the SO_3^{2-} was oxidized to SO_4^{2-}.

Effects of thiol binding reagents on RuBP carboxylase

Thiol-group binding reagents (*p*-hydroxymercuribenzoate [*p*HMB) and *N*-ethylmaleimide (NEM)] strongly inhibited RuBP carboxylase activity (Table 2). The addition of excess dithio erythritol (DTE) protected the enzyme from the inhibitory effect of NEM, indicating that sulphydryl groups of the enzyme were essential for enzyme activity. The obligatory involvement of sulphydryl groups for structural conformation of this enzyme protein has also been shown for spinach and *Chromatium* enzymes [28, 29].

Table 2. Inhibition of RuBP carboxylase by thiol binding compounds and its reversal by DTE

Treatment	Enzyme activity (units/mg protein)
Control	1.66
+ *p*HMB (4 mM)	0.01
+ NEM (2 mM)	0.57
+ NEM (4 mM)	0.12
+ NEM (2 mM) + DTE (10 mM)	1.69

Characterization of glycollate oxidase from P. banksiana

As with RuBP carboxylase, the activity of glycollate oxidase also increased (70–80%) during greening and maturation of the needles. Mature needles were therefore used in all experiments. The enzyme showed optimum activity at pH 8 (Tris buffer), and the rate of reaction was linear up to 15 min. The extraction of enzyme was best achieved in a phosphate buffer—PVP-10 medium similar to that described for RuBP carboxylase. The enzyme from the 120000 *g* supernatant was partially purified by fractionation with ammonium sulphate. Most of the activity (80%) was precipitated at 25–50% ammonium sulphate saturation, resulting in a 50–60% increase in specific activity over that of the supernatant. The enzyme was stable for several days when stored in a frozen condition. The enzyme showed hyperbolic reaction kinetics with respect to its substrate, glycollic acid (K_m was 36 mM).

Effects of SO_3^{2-} and SO_4^{2-} on glycollate oxidase

Glycollate oxidase was much more sensitive to SO_3^{2-} than was RuBP carboxylase. More than 50% inhibition was observed at 10 μM SO_3^{2-} (Table 3), and the inhibition became more severe at higher concentrations. SO_3^{2-} inhibited the enzyme competitively with respect to glycollic acid (K_i for SO_3^{2-} was 5.7 μM). Glycollate oxidase from spinach has also been shown to be very sensitive to SO_3^{2-} [30, 31] due to the formation of glyoxylate bisulphite, a potent competitive inhibitor of the enzyme [31]. The addition of up to 2.5 mM SO_4^{2-} did not produce any appreciable effect on the enzyme activity (Table 3).

Effects of sulphur dioxide fumigation on RuBP carboxylase and glycollate oxidase

Accumulation of SO_3^{2-} and SO_4^{2-} in plant leaves is known to occur upon sulphur dioxide fumigation [32]. Above a certain concentration the absorbed sulphur dioxide (or its accumulation products) is expected to affect sensitive metabolic processes. This was tested by fumigating pine seedlings with 0.34 ppm gaseous sulphur dioxide and measuring its effect on the activities of RuBP carboxylase and glycollate oxidase. Fumigation for 1 hr did not produce any change in the activities of these enzymes (Table 4). This lack of response could be due to: (a) rapid air movement (as a result of a high airflow rate) that caused little or no change in the carbon dioxide level in the cuvette air; (b) the presence of active SO_3^{2-} oxidizing systems in the needles [Khan, A. A. and Malhotra, S. S., unpublished results]; and (c) the reduction of initially absorbed sulphur dioxide (or its oxidation products) in the chloroplasts either to the normal metabolic products of the reductive sulphur cycle or to hydrogen sulphide and its release from the needle tissues [32].

Table 3. Effects of SO_3^{2-} and SO_4^{2-} on glycollate oxidase

SO_3^{2-} (μmol)	Enzyme activity (units/mg protein)	SO_4^{2-} (mmol)	Enzyme activity (units/mg protein)
0	0.188	0	0.172
2.5	0.168	1.0	0.172
5.0	0.120	2.5	0.167
10.0	0.086		

Table 4. Effects of sulphur dioxide fumigation (0.34 ppm) on RuBP carboxylase and glycollate oxidase activities and sulphur content of fumigated pine needles

Duration of SO_2 fumigation* (hr)	Enzyme activity		
	RuBP carboxylase	Glycollate oxidase (% control)	Sulphur†
1	100.0	100.0	100.5
24	88.9	91.3	165.0
48	70.8	76.6	200.0

*As described in the text, plants were fumigated with an 18 hr photoperiod/day.

†The sulphur content in the unfumigated needles was 1.00 ± 0.2 mg/g dry wt of needles.

Rapid airflow in the cuvette is expected to keep the carbon dioxide level in plant tissues high enough to maintain RuBP carboxylase in its activated form. Furthermore, the amount of initially absorbed sulphur dioxide in the tissues would be considerably lower than the amount of carbon dioxide (due to a large difference in carbon dioxide and sulphur dioxide levels in the cuvette air). This would, therefore, limit the availability of carbon dioxide-binding sites for sulphur dioxide action. Stomatal closure that generally occurs at high carbon dioxide levels [32] may also be another factor that restricts initial sulphur dioxide absorption.

The presence of SO_3^{2-} oxidizing systems in the needles would rapidly convert small amounts of toxic species of dissolved sulphur dioxide (SO_3^{2-} and HSO_3^-) to a relatively less toxic form, SO_4^{2-}. It has been reported that SO_4^{2-} is relatively harmless to photosynthesis [33, 34]. Both RuBP carboxylase and glycollate oxidase in *P. banksiana* were found to be less sensitive to SO_4^{2-} than to SO_3^{2-}; therefore, it would appear that SO_3^{2-} toxicity depends on the efficiency of the SO_3^{2-} oxidizing system of the tissues. Furthermore, the changes in the tissue balance of sulphydryl and disulphide groups in the fumigated plant tissues [32] can affect the activities of enzymes requiring these groups.

Fumigation of intact plants of *Glycine max* with $^{35}SO_2$ has been shown to result in the accumulation of mainly $^{35}SO_4^{2-}$ [35]. In our experiment (1 hr fumigation), the pine needles probably did not accumulate sufficient quantities of SO_3^{2-} to be toxic. This is supported by the fact that the sulphur content of the needles did not increase after the exposure. Both enzymes, however, were markedly inhibited after exposure of pine seedlings to sulphur dioxide for 24 and 48 hr (Table 4). These exposures did not produce any visible symptoms of injury. The inhibitory effect was accompanied by an increase in the sulphur content of the needles, perhaps indicating an accumulation of toxic levels of SO_3^{2-} and SO_4^{2-} in the tissues. The decrease in RuBP carboxylase and glycollate oxidase therefore appeared to be related to increased sulphur dioxide uptake and the sensitivity of the enzymes to accumulated SO_3^{2-} and SO_4^{2-}. Glyoxylate bisulphite, a competitive inhibitor of glycollate oxidase, has been shown to accumulate in rice plants after large doses of $^{35}SO_2$ [36]. If small amounts of glyoxylate bisulphite are continuously formed during long sulphur dioxide exposures, it would affect glycollate oxidase and its dependent processes. For example, glycollic acid is known to affect stomatal opening in light [37], which in turn affects photosynthesis.

EXPERIMENTAL

Plant growth. Jack pine (*Pinus banksiana* Lamb.) seedlings were grown in peat moss in styroblock trays [38] placed in a greenhouse at 20–24° under natural daylight supplemented by 12 klx fluorescent light (16 hr). Mature needles from 4–5-month-old plants were used.

Fumigation of plants with SO_2. Plants were transferred from the styroblock trays to fumigation cuvettes as described previously [39]. The construction and operation of the cuvettes were essentially the same as described before [39] except that the cuvettes used in the present study were modified for a high airflow rate (*ca* 100 l/min).

For each expt, sets of *ca* 12 seedlings of uniform age and growth were transferred to two cuvettes. One cuvette received clean air (control), while the other cuvette received air containing 0.34 ppm SO_2. Prior to fumigation, the cuvettes containing the plants were placed in a controlled environment chamber for 48 hr and were maintained at 20 klx light intensity (18 hr photoperiod). The temp. in light and dark periods was 22 and 18°, respectively. Relative humidity inside the cuvettes was maintained at 60–65% by using prehumidified air. The rate of airflow in the cuvettes was 100 l/min. During fumigation the concr of SO_2 was maintained at 0.34 ppm by a feedback controller and was continuously monitored by a Phillips PW 9700 SO_2 analyser. A fan at the inlet port of each cuvette provided uniform air mixing. Plants were harvested after fumigation, and the mature needles were used for experimental purposes.

Extraction and purification of enzymes. Needles (1 cm sections) were homogenized for 1 min with a Brinkman Polytron Homogenizer (Model PT-10) at full power in 10 vol. of a chilled soln of 0.1 M KPi buffer (pH 7.5) containing 1% PVP-10, 1 mM DTE, and 1 mM $MgCl_2$. The homogenate was filtered through Miracloth and centrifuged at 120000 *g* for 30 min. The supernatant was then used for the purification of RuBP carboxylase and glycollate oxidase.

RuBP carboxylase was purified from the 120000 *g* supernatant by an initial fractionation with $(NH_4)_2SO_4$. The enzyme was pptated by adding solid $(NH_4)_2SO_4$ to the supernatant to a 60% satn point. The pptated proteins were removed by centrifugation, dissolved in 0.1 M Tris buffer (pH 7.5), and desalted by passing through a small column (2 × 15 cm) of Biogel P-2. The enzyme was then further purified by gel filtration on Sepharose 6B columns (2 × 45 cm). Equilibration and elution of the columns was done with 0.1 M Tris buffer (pH 7.5); 3.5 ml enzyme fractions were collected and analysed for enzyme activity and protein content.

Glycollate oxidase was partially purified by precipitating the enzyme with $(NH_4)_2SO_4$. The protein fraction obtained at 25–50% satn was dissolved in 0.1 M Tris buffer (pH 7.5) and used in the enzyme reaction.

For both RuBP carboxylase and glycollate oxidase purifications, all of the above operations were carried out at 0–4°. In the fumigation expts, the enzyme activity was assayed in the 120000 *g* supernatant because the change in enzyme activities was similar whether they were assayed after partial purification or in the 120000 *g* supernatant.

Enzyme assays. RuBP carboxylase. A regular assay in a final vol. of 0.2 ml involved preincubation of the enzyme for 3 min at 30° in a reaction medium containing 10 μmol Tris buffer (pH 7.5), 2 μmol $MgCl_2$ and 4 μmol [^{14}C]$NaHCO_3$ (9.8×10^5 cpm). Where indicated, specified test compounds were included in the preincubation period. The enzyme reaction was started by adding 150 nmol of RuBP. After 1–3 min at 30° the reaction was terminated by adding 0.1 ml HCO_2H (88%). In some kinetic expts (Fig. 2), the activity of the purified enzyme was assayed according to the procedure outlined in ref. [22] with some modifications. This procedure involved activation of the enzyme by preincubating it for 3 min in 10 mM [^{14}C]$NaHCO_3$ and 20 mM $MgCl_2$ at 30°. The activated form of the enzyme was then used to start the reaction under N_2 gas. The assay mixture in a final vol of 0.2 ml contained 10 μmol Tris buffer (pH 7.5), 2 μmol $MgCl_2$, varying concns of [^{14}C]$NaHCO_3$, 150 nmol RuBP, activated enzyme, and H_2O or a specified amount of SO_3^{2-} (in assays involving SO_3^{2-} effect). The amount of HCO_3^- present in the enzyme aliquot was taken into account in calculating its total concr in each assay. Thr reaction was run for 3 min at 30° and stopped by adding 0.1 ml HCO_2H (88%). An aliquot from either procedure was then transferred to a 7-ml scintillation vial, and the contents of the vial were flushed with N_2 gas to almost dryness on a hot-water bath. Incorporation of ^{14}C into acid-stable products was then determined by dissolving the contents of the vial in 0.1 ml H_2O and mixing them with 5 ml of a scintillation fluid [0.4% Omnifluor (New England Nuclear) dissolved in 30% EtOH in toluene]; counting was done with a liquid scintillation spectrometer. A reaction control in which RuBP was not added during the assay was always run and the counts, if any, were subtracted from those of the treatment assays. A unit of enzyme activity was defined as 1 μmol HCO_3^- incorporated into acid-stable products/min under assay conditions. *Glycollate oxidase.* Enzyme activity was assayed according to ref. [40] with some modifications. The assay system in a final vol. of 2 ml contained 100 μmol Tris buffer (pH 8.0), 15 μmol glycollic acid (pH adjusted to 7.0), 5 μmol phenylhydrazine HCl (pH adjusted to 7.0), enzyme soln and H_2O. The reaction was run for 1–10 min. The increase in A_{324nm} was followed, using a molar extinction coefficient of 17×10^3/cm/mol [41] for calculating the activity. A unit of enzyme activity was defined as 1 μmol glyoxyllate for formed/min under assay conditions. *Other estimations.* Protein was estimated according to ref. [42] after pptation with TCA and washing the ppt with 80% Me_2CO. Bovine serum albumin was used as a standard. Needle dry wt was measured by oven-drying the fresh needles at 80° for 24 hr. For S analysis the dried samples were powdered and a portion was used for O_2-flask combustion [43] and S was measured according to ref. [44].

All expts were repeated several times and the trends were found to be highly reproducible. The data in the tables are taken from representative expts.

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REFERENCES

1. Ku, M. B. S., Schmitt, M. R. and Edwards, G. E. (1979) *J. Exp. Botany* **30**, 89.
2. Wareing, P. F., Khalifa, M. M. and Treharne, K. J. (1968) *Nature (London)* **220**, 453.
3. Bjorkman, O. (1968) *Physiol. Plant.* **21**, 1.
4. Anderson, W. R., Wildner, G. F. and Criddle, R. S. (1970) *Arch. Biochem. Biophys.* **137**, 84.
5. Bowes, G., Orgen, W. L. and Hageman, R. H. (1971) *Biochem. Biophys. Res. Commun.* **45**, 716.
6. Andrews, T. G., Lorimer, G. H. and Tolbert, N. E. (1973) *Biochemistry* **12**, 11.
7. Lorimer, G. H., Andrews, T. G. and Tolbert, N. E. (1973) *Biochemistry* **12**, 18.
8. Thomas, M. D. and Hill, G. R. (1937) *Plant Physiol.* **12**, 309.
9. Oshima, Y., Ushijima, T. and Tazaki, T. (1973) *Environ. Control Biol.* **11**, 103.
10. Bull, J. N. and Mansfield, T. A. (1974) *Nature (London)* **250**, 443.
11. Black, V. G. and Unsworth, M. H. (1979) *J. Exp. Botany* **30**, 473.
12. Furukawa, A., Natori, T. and Tatsuka, T. (1980) *Natl. Inst. Environ. Stud., Jpn, Res. Rep.* **11**, 1.
13. Malhotra, S. S., Addison, P. A. and Khan, A. A. (1980) in *Research Report, LS* 3.1. Alberta Oil Sands Environmental Research Program, Edmonton.
14. Koziol, M. J. and Cowling, D. W. (1978) *J. Exp. Botany* **29**, 1431.
15. Hallgren, J. E. (1978) in *Sulfur in the Environment, Part II: Ecological Impacts* (Nriagu, J. O., ed.) p. 163. John Wiley, New York.
16. Chollet, R. and Anderson, L. L. (1977) *Biochim. Biophys. ACTA* **482**, 228.
17. Hurner, N. P. A. and Macdowall, F. D. H. (1978) *Can. J. Biochem.* **56**, 1154.
18. Pon, N. G., Rabin, B. R. and Calvin, M. (1963) *Biochem. Z.* **338**, 7.
19. Tabita, F. R. and McFadden, B. A. (1974) *J. Biol. Chem.* **249**, 3453.
20. Lorimer, G. H., Badger, M. R. and Heldt, H. W. (1978) in *Photosynthetic Carbon Assimilation* (Siegelman, H. W. and Hind, G., eds.) p. 283. Plenum Press, New York.
21. Paech, C., McCurry, S. D., Pierce, J. and Tolbert, N. E. (1978) in *Photosynthetic Carbon Assimilation* (Siegelman, H. W. and Hind G., eds.) p. 227. Plenum Press, New York.
22. Andrews, T. J., Badger, M. R. and Lorimer, G. H. (1975) *Arch. Biochem. Biophys.* **171**, 93.
23. Badger, M. R. and Lorimer, G. H. (1976) *Arch. Biochem. Biophys.* **175**, 723.
24. Laing, W. A. and Christeller, J. T. (1976) *Biochem. J.* **159**, 563.
25. Gezelius, K. and Hallgren J. E. (1980) *Physiol. Plant.* **49**, 354.
26. Ziegler, I. (1972) *Planta* **103**, 155.
27. Trown, P. W. (1965) *Biochemistry* **4**, 908.
28. Sugiyama, T., Nakayama, N., Ogawa, M., Akawaza, T. and Oda, T. (1968) *Arch. Biochem. Biophys.* **125**, 98.
29. Takabe, T. and Akazawa, T. (1975) *Arch. Biochem. Biophys.* **169**, 686.
30. Zelitch, I. (1957) *J. Biol. Chem.* **224**, 251.
31. Zelitch, I. (1958) *J. Biol. Chem.* **233**, 1299.
32. Malhotra, S. S. and Khan, A. A. (1982) in *Air Pollution and Plant Life* (Treshow, M., ed.). John Wiley, London (in press).
33. Hill, D. J. (1974) *New Phytol.* **73**, 1193.
34. Ferguson, P. and Lee, J. A. (1979) *New Phytol.* **82**, 703.
35. Garsed, S. G. and Read, D. J. (1977) *New Phytol.* **99**, 583.
36. Tanaka, H., Takanashi, T. and Yatazawa, M. (1972) *Water Air Soil Pollut.* **1**, 205.
37. Zelitch, I. and Walker, D. A. (1964) *Plant Physiol.* **39**, 856.
38. Malhotra, S. S. (1976) *New Phytol.* **76**, 239.
39. Malhotra, S. S. and Khan, A. A. (1978) *Phytochemistry* **17**, 241.
40. Hess, J. L. and Tolbert, N. E. (1967) *Plant Physiol.* **42**, 371.
41. Dixon, G. H. and Kornberg, H. L. (1959) *Biochem. J.* **72**, 3.
42. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.
43. Chan, C. C. Y. (1975) *Analyt. Letters* **8**, 655.
44. Carson, J. A., Crepin, J. M. and Nemunis Siugzdines, P. (1972) *Can. J. Soil Sci.* **52**, 278.

PEROXIDASE ACTIVITY AS AN INDICATOR OF SO_2 INJURY IN JACK PINE AND WHITE BIRCH

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Peroxidase Activity as an Indicator of SO_2 Injury in Jack Pine and White Birch

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Key Term Index: peroxidase, sulphur uptake, sulphur dioxide effects; *Pinus banksiana; Betula papyrifera*

Summary

Peroxidase (EC 1.11.1.7) activity in jack pine *(Pinus banksiana)* and white birch *(Betula papyrifera)* seedlings increased greatly during aging of the foliar tissues. Fumigation of seedlings with SO_2 caused a marked increase in enzyme activity and sulphur content of both species; these responses were, however, more pronounced in white birch than in jack pine. The stimulation of peroxidase in SO_2-fumigated jack pine seedlings appeared to be due to increased production of the peroxidase isoenzymes rather than their activation. Increase in both peroxidase activity and sulphur content in the fumigated needle tissues appeared to be transitory in nature as shown by their decline during recovery in an SO_2-free atmosphere. A possible role of peroxidase in SO_2-initiated free radical process is discussed.

Introduction

Considerable research has been reported on the impact of air pollutants on various plant enzyme systems (Malhotra and Khan 1982). This has enabled elucidation of the mechanisms of pollutant toxicity in some cases along with the development of biochemical techniques to detect air pollution injury to vegetation prior to the development of visible symptoms of phytotoxicity.

A number of air pollutants such as ozone (Curtis and Howell 1971; Curtis et al. 1976; Tingey et al. 1976), fluoride (Keller 1974), nitrogen dioxide (Horsman and Wellburn 1975) and sulphur dioxide (Keller 1976; Keller et al. 1976; Horsman and Wellburn 1975, 1977) cause an increase in peroxidase (EC 1.11.1.7) activity in a variety of plant tissues. Several theories explaining such a response have been advanced. Howell and Kremer (1973) suggested that peroxidase stimulates the formation of quinones which subsequently polymerize with proteins and amino acids to form the dark pigments, characteristic of damaged leaves. It has also been proposed that peroxidase stimulation accelerates the aging process (Keller and Schwager 1971) since normally senescing leaves also contain high levels of peroxidase activity.

Although it is known that peroxidase activity is stimulated in plants exposed to SO_2, it is not certain whether this response is due to activation or increased de novo synthesis of the enzyme. No data are available to show a relationship between increased peroxidase activity and SO_2 uptake in the fumigated plant tissues. Furthermore, response of peroxidase activity and sulphur uptake upon transferring the SO_2-fumigated plants to a SO_2-free atmosphere has not been demonstrated. In the present investigation, these responses were studied using primarily SO_2-fumigated jack pine seedlings. In SO_2-fumigated white birch seedlings, only the response of peroxidase in relation to leaf sulphur uptake was investigated.

Materials and Methods

Plant growth

Jack pine (*Pinus banksiana* Lamb.) seedlings were grown in greenhouse conditions as described previously (Malhotra and Khan 1980). Six-mon-old seedlings were used for experimental purposes. The needles were divided into young mature (3-mon) and old (6-mon) tissues.

White birch (*Betula papyrifera* Marsh.) seeds were planted in small plastic pots (4") containing peat. The remainder of the growth conditions were the same as for jack pine. The leaves from the three-mon-old seedlings were divided into young mature (top 4 leaves) and old (below the top 4 leaves) tissues.

Fumigation conditions

Prior to fumigation, soil in the pots containing birch seedlings was covered with parafilm to avoid SO_2 absorption by moist soil. Jack pine and white birch seedlings were fumigated with SO_2 in microchambers using a continuous flow-through system as described previously (Malhotra and Khan 1980); the flow of the air in the microchamber was adjusted to 100 l/min.

After the treatments the foliage from both species was harvested and separated into the age groups described above, and only the young mature tissue was analysed.

Enzyme extraction

Peroxidase was extracted from the foliage using a modified method of Keller and Schwager (1971). Midribs were removed from the leaves and the foliar tissue was cut into small pieces. The excised needle or leaf tissue (0.3 g) was then homogenized for 1 min in 25 ml of cold 0.1 M K-phosphate buffer (*p*H 7.0) with a Brinkman Polytron homogenizer (Model PT-10). The homogenizer probe was rinsed with 25 ml cold distilled water. The rinse and the homogenate were combined and filtered through Miracloth. The filtrate was centrifuged at 12,000 · g for 30 min at 0—4 °C and the supernatant was used as a "crude enzyme extract".

Enzyme purification

The "crude enzyme extract" from jack pine needles was subjected to ammonium sulphate precipitation initially to a 40% saturation. After stirring for 1 h the suspension was centrifuged at 12,000 · g for 10 min. The supernatant was then brought to an 85% ammonium sulphate saturation; after 1 h stirring it was centrifuged at 12,000 · g for 10 min. The protein precipitate thus obtained was dissolved in 10 ml of 0.1 M K-phosphate buffer (*p*H 7.0). This protein fraction contained most of the peroxidase activity, and was used as "partially purified enzyme". All the above operations were carried out at 0 –4 °C.

Enzyme assay

The reaction was carried out in spectrophotometric cuvettes containing 200 μmoles K-phosphate buffer (*p*H 7.0), 46 μmoles p-phenylenediamine, appropriately diluted enzyme extract and distilled water to a final volume of 2.9 ml. The reaction was started by mixing 0.1 ml of 0.6% H_2O_2 to the reaction cuvette.

The rate of the reaction was followed at room temperature (22 – 24 °C) by recording optical density increase at 485 nm with a Carl Zeiss spectrophotometer (Model M4Q 111) coupled to a high voltage Photometer Indicator Unit. A unit of enzyme activity was expressed as the amount of enzyme producing an increase of 0.001 O.D./min under the assay conditions. A control assay in which H_2O_2 was omitted during the reaction was run simultaneously and the rate if any was subtracted from the total enzyme rate. In all the assays the rate without added H_2O_2 was very low.

Polyacrylamide gel electrophoresis

Electrophoresis of "partially purified enzyme" and "crude enzyme" extracts was carried out according to the procedure of Davis (1964). Polyacrylamide gels were prepared by mixing 15 ml of acrylamide solution (13.65% acrylamide and 0.35% N, N'-methylenebisacrylamide) with 0.1 ml of N,N,N',N'-tetramethylethylenediamine (TMED), 15 ml of 0.04 M Tris-glycine buffer (*p*H 8.3) and 0.7 ml of 1% ammonium persulphate. The electrophoresis was conducted at room temperature using 0.04 M Tris-glycine buffer (*p*H 8.3) with a constant current of 2.0 mA per gel. The peroxidase bands in the gels were detected by a modified benzidine-H_2O_2 method (Novacky and Hampton 1968).

Other estimations

For dry weight a sample of the fresh foliage was oven dried at 80 °C for 24 h. For sulphur analysis the dried sample was ground to a fine powder and combusted using Oxygen-Flask method (Chan 1975). The total sulphur in the sample was then determined by a modification of Johnson-Nishita technique (Carson et al. 1972). Protein content of the enzyme extracts was measured according to Lowry et al. (1951).

Results and Discussion

Each sample comprised a mixture of foliage from 6 or more seedlings; the experimental data, therefore, represent averages of populations. The experiments for each investigation were conducted several times and their trends were found to be reproducible.

Peroxidase activity from both jack pine and white birch foliage was easily and completely extracted with 0.1 M K-phosphate buffer (*p*H 7.0). The enzyme showed a broad *p*H-optima in the range of *p*H 6.0—7.5, and was stable upon storage in cold. At limiting concentrations of the enzyme in the assays the rate of the reaction was proportional to the concentrations, and the reaction time (up to 5 min). In all the experiments the enzyme activity was followed under optimum assay conditions.

Enzyme activity in different-aged tissues

Peroxidase activity in the foliage of both jack pine and white birch seedlings was markedly higher in the old tissues than in the young mature ones (Table 1). Increase in peroxidase activity with aging has been shown in other plants (Keller and Schwager 1971; Grill et al. 1980). Increased peroxidase activity has been implicated in leaf senescence mainly because of its ability to oxidize indoleacetic acid, a growth hormone. It is possible that in senescing old tissues the rise in peroxidase activity is related to increased production of H_2O_2 (Birecka et al. 1979).

Polyacrylamide gel electrophoresis of the peroxidase preparations from the two age groups of jack pine needles revealed that both types of needles contain the same isoenzyme pattern, and that the increased activity in the old tissues was due to increased synthesis of the same isoenzymes. In *Larix decidua*, however, increased peroxidase activity during seasonal growth, appeared to be related to the synthesis of new isoenzymes (Grill et al. 1980).

Table 1. *Peroxidase activity in different-aged foliage of jack pine and white birch seedlings*

Plant species	Peroxidase activity (units/g dry wt.)	
	Young mature	old
Jack pine	59.1	207.7
White birch	101.3	153.8

Effects of SO_2-fumigation on peroxidase activity and sulphur uptake by foliage

Fumigation of jack pine seedlings with various concentrations and durations of SO_2 exposure markedly affected peroxidase activity and sulphur content of the needle tissues (Fig. 1). At a low SO_2 concentration of 0.17 ppm, the peroxidase activity and sulphur content remained unchanged initially (4 days), then rose by 50–60% between 4th and 7th day, after which no further change occurred for the remainder of the fumigation period. At 0.34 ppm SO_2, the response in both peroxidase activity and sulphur uptake was almost parallel; the effect was, however, markedly higher at

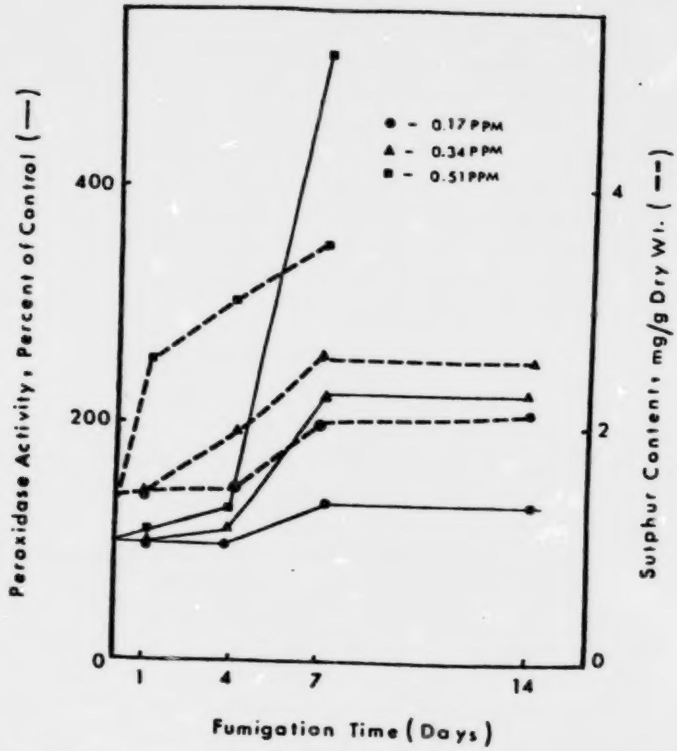

Fig. 1. *Effect of SO_2-fumigation on peroxidase activity and sulphur content of jack pine needles.*

Table 2. *Effect of gaseous SO_2 on peroxidase activity and sulphur content of white birch leaves*

Treatment	Duration (days)	Peroxidase activity (% of control)	Sulphur content (mg/g dry wt.)
Control		100.0	1.68
0.17 ppm SO_2	7	220.6	4.28
0.34 ppm SO_2	3	196.4	6.93

0.34 ppm than at 0.17 ppm SO_2. Exposure to 0.51 ppm SO_2, produced a dramatic increase in both processes. In general, during the initial period of fumigation, the effect was greater on sulphur uptake than on peroxidase activity. After Day 4 of fumigation, the stimulation of peroxidase activity is suggested to be due to a parallel increase in sulphur uptake by the tissues which eventually resulted in the development of visible symptoms of SO_2 toxicity in the foliage. Chlorotic symptoms of SO_2 toxicity were observed only in plants exposed to 0.51 ppm SO_2 after 7 days of fumigation. Similar types of chlorotic symptoms have been reported on various forest species exposed to SO_2 (Malhotra and Blauel 1980).

SO_2 fumigation of white birch seedlings also stimulated peroxidase activity with a parallel increase in sulphur content of the leaves. White birch, however, appeared to be more sensitive to SO_2 than jack pine as evidenced by a marked increase in peroxidase activity and sulphur uptake even at a low (0.17 ppm) SO_2 concentration (Table 2). An increase in SO_2 concentration (0.34 ppm) caused a rapid increase in both peroxidase activity and sulphur content of the tissues; the fumigated plants showed severe chlorotic symptoms after 3 days of SO_2 fumigation.

Results from these experiments clearly indicate that in both species the cellular responses and the resulting visible injury were dependent on SO_2 concentration and duration of exposure. Similar effects have been summarized on various biochemical and physiological processes of plants (Malhotra and Khan 1982).

Increase in the sulphur content of the tissues as a result of either acute or chronic fumigation with SO_2 suggests that the fumigated tissues may have accumulated sulphur mainly in the forms of SO_3^{2-}, HSO_3^-, and SO_4^{2-}. These species are known to accumulate in plants exposed to SO_2, and are highly phytotoxic to many cellular processes (Malhotra and Khan 1982). Oxidation of SO_3^{2-} and HSO_3^- by free radical mechanisms produces superoxide radical ($\bar{O}_2$), which is converted to H_2O_2 by either spontaneous or superoxide dismutase catalyzed reactions (Asada 1980). Plant leaves with high levels of superoxide dismutase, an enzyme which is induced by low SO_2 concentrations, have been shown to be resistant to SO_2 (Tanaka and Sugahara 1980). It would follow from this that SO_2 fumigation would result in an increased conversion of highly toxic $\bar{O}_2$ to H_2O_2, and the accumulation of the latter in the tissues. Since H_2O_2 is also very toxic, the stimulation of the peroxidase activity by SO_2 fumigation may be a cellular defence mechanism to eliminate toxic levels of H_2O_2.

Stimulation of peroxidase activity in SO_2-fumigated plants could be either due to an activation of the enzyme (non-cellular response) or due to its increased synthesis

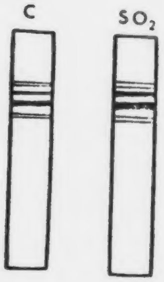

Fig. 2. *Peroxidase isoenzymes in control (C) — and SO_2-fumigated (SO_2) jack pine needles. Dark bands — major isoenzymes (dense staining with benzidine-H_2O_2); open bands — minor isoenzymes (light staining with benzidine-H_2O_2).*

(cellular response). Incubation of the isolated enzyme extracts with various concentrations of aqueous SO_2 (0.5 mM to 2.5 mM) for up to 4 h produced no increase in peroxidase activity, suggesting that under in vivo conditions SO_2 was not acting as an activator of the enzyme. The in vivo stimulation of the peroxidase activity in the SO_2-fumigated plants, therefore, appears to be due to an increased cellular synthesis of the enzyme protein. This process may involve a production of either new species of isoenzymes or an increased synthesis of those already present in the tissues. This was tested by comparing the isoenzyme pattern and concentrations in control and fumigated plant extracts of jack pine needles. Polyacrylamide gel electrophoresis, using identical protein aliquots from control and SO_2-fumigated tissues, showed that the isoenzyme pattern remained unchanged after SO_2 treatment (Fig. 2): the SO_2-fumigated seedlings, however, contained higher levels of each isoenzyme than those in control seedlings. This supports the suggestion that the increased peroxidase activity in SO_2-fumigated plants was due to an increased peroxidase synthesis triggered by altered metabolic events in the SO_2-fumigated tissues.

Increased peroxidase activity upon air pollution stress has been suggested to be an indicator of previsible cellular injury (KELLER 1974). There are, however, no reports on the reversibility of this response upon transferring the SO_2-fumigated plants

Table 3. *Changes in peroxidase activity and sulphur content of SO_2-fumigated jack pine needles following a SO_2-free recovery period*

Treatment	Duration (days)	Recovery (days)	Peroxidase activity		Sulphur content (mg/g dry wt.)
			(units/g dry wt.)	(% of control)	
Control	—	—	59.6	100	1.34
0.34 ppm SO_2	7	0	146.5	246	2.69
0.34 ppm SO_2	7	21	109.8	184	1.13
0.51 ppm SO_2	7	0	304.7	511	3.10
0.51 ppm SO_2	7	21	93.5	157	1.84

to SO_2-free atmosphere. We, therefore, studied the nature of such a response in needles of pine seedlings exposed to two concentrations of SO_2. The results in Table 3 showed that increased peroxidase activity and sulphur content of the SO_2-fumigated seedlings greatly decreased during the SO_2-free recovery period. This indicates a transitory (reversible) nature of the response. A marked decrease in the sulphur content of the SO_2-fumigated plant tissues during the recovery period also indicated that the sulphur absorbed during the fumigation was either metabolized and lost from the tissues or was translocated to other parts of the plant. Using labeled SO_2 [$^{35}SO_2$] it has been shown that the absorbed SO_2 does not remain fixed at the site of absorption but can be translocated to other parts of the plant (Jensen and Kozlowski 1975; Garsed and Read 1977). These results are, therefore, of considerable importance for interpretation of SO_2 effects on vegetation.

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References

Asada, K.: Formation and scavenging of superoxide in chloroplasts, with relation to injury by sulfur dioxide. Res. Rep. Natl. Environ. Stud. Japan **11**, 165—169 (1980).

Birecka, H., Chaskes, M. J., and Goldstein, J.: Peroxidase and senescence. J. Exp. Bot. **30**, 565—573 (1979).

Carson, J. A., Crepin, J. M., and Nemunis-Siugzdines, P.: A sulphate-sulphur method used to delineate the sulphur status of soils. Can. J. Soil Sci. **52**, 278—281 (1972).

Chan, C. C. Y.: Determination of total sulphur in vegetation by a turbidimetric method following an oxygen-flask combustion. Anal. Lett. **8**, 655—663 (1975).

Curtis, C. R., and Howell, R. K.: Increase in peroxidase isoenzyme activity in bean leaves exposed to low doses of ozone. Phytopathology **61**, 1306—1307 (1971).

Curtis, C. R., Howell, R. K., and Kremer, D. F.: Soybean peroxidases from ozone injury. Environ. Pollut. **11**, 189—194 (1976).

Davis, B. J.: Disc electrophoresis. II, Method and application to human serum proteins. Ann. N. Y. Acad. Sci. **121**, 404—427 (1964).

Garsed, S. G., and Read, D. J.: The uptake and metabolism of $^{35}SO_2$ in plants of differing sensitivity to sulphur dioxide. Environ. Pollut. **13**, 173—186 (1977).

Grill, D., Esterbauer, H., and Birkner, M.: Untersuchungen über die Peroxidaseaktivität in Larchennadeln. Beitr. Biol. Pflanzen **55**, 67—76 (1980).

Horsman, D. C., and Wellburn, A. R.: Synergistic effects of SO_2 and NO_2 polluted air upon enzyme activity in pea seedlings. Environ. Pollut. **8**, 123—133 (1975).

Horsman, D. C., and Wellburn, A. R.: Effect of SO_2 polluted air upon enzyme activity in plants originating from areas of different annual mean atmospheric SO_2 concentrations. Environ. Pollut. **13**, 33—39 (1977).

Howell, R. K., and Kremer, D. F.: The chemistry and physiology of pigment in leaves injured by air pollution. J. Environ. Qual. **2**, 434—438 (1973).

Jensen, K. F., and Kozlowski, T. T.: Absorption and translocation of sulphur dioxide by seedlings of four forest tree species. J. Environ. Qual. **4**, 379—382 (1975).

KELLER, TH.: The use of peroxidase activity for monitoring and mapping air pollution areas. Eur. J. For. Pathol. **4**, 11—19 (1974).

KELLER, TH.: Auswirkungen niedriger SO_2-Konzentrationen auf junge Fichten. Schweiz. Z. Forstwes. **127**, 237—251 (1976).

KELLER, TH., und SCHWAGER, H.: Der Nachweis unsichtbarer („physiologischer") Fluor-Immissionschädigungen an Waldbäumen durch eine einfache kolorimetrische Bestimmung der Peroxidase-Aktivität. Eur. J. For. Pathol. **1**, 6—18 (1971).

KELLER, TH., SCHWAGER, H., und YEE-MEILER, D.: Der Nachweis winterlicher SO_2-Immissionen an jungen Fichten. Ein Vergleich dreier Methoden. Eur. J. For. Pathol. **6**, 244—249 (1976).

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J.: Protein measurement with the folin phenol reagent. J. Biol. Chem. **193**, 265—275 (1951).

MALHOTRA, S. S., and BLAUEL, R. A.: Diagnosis of air pollutant and natural stress symptoms on forest vegetation in Western Canada, Environ. Can., Can. For. Serv., North. For Res. Cent. Inf. Rep. NOR-X-228 (1980).

MALHOTRA, S. S., and KHAN, A. A.: Effects of sulphur dioxide and other air pollutants on acid phosphatase activity in pine seedlings. Biochem. Physiol. Pflanzen 175, 228—236 (1980).

MALHOTRA, S., and KHAN, A. A.: Biochemical and physiological impact of major pollutants. In: "Atmospheric pollution and plant life" (Edit. TRESHOW, M.), John Wiley and Sons Ltd. London, In press (1982).

NOVACKY, A., and HAMPTON, R. E.: Peroxidase isoenzymes in virus-infected plants. Phytopathology 58, 301—305 (1968).

TANAKA, K., and SUGAHARA, K.: Role of superoxide dismutase in the defense against SO_2 toxicity and induction of superoxide dismutase with SO_2 fumigation. Res. Rep. Natl. Inst. Environ. Stud. Japan **11**, 155—164 (1980).

TINGEY, D. T., FITES, R. C., and WICKLIFF, C.: Differential foliar sensitivity of soybean cultivars to ozone associated with differential enzyme activities. Physiol. Plant. **37**, 69—72 (1976).

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PROTEIN BIOSYNTHESIS IN JACK PINE AND ITS INHIBITION BY SULPHUR DIOXIDE

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Phytochemistry

PROTEIN BIOSYNTHESIS IN JACK PINE AND ITS INHIBITION BY SULPHUR DIOXIDE

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Key Work Index--*Pinus banksiana; Pinaceae*; jack pine; SO_2 effects; protein biosynthesis

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Abstract--Incorporation of [U-^{14}C] leucine in jack pine (*Pinus banksiana* Lamb.) occurred mainly in the chloroplast and soluble cytoplasmic fractions. In the chloroplasts a major portion of the label was associated with the membrane-bound proteins. Fumigation of pine seedlings with gaseous SO_2 (0.34 ppm) for 24 and 48 hr markedly inhibited *de novo* protein biosynthesis in the chloroplast and cytoplasmic fractions. The inhibition was greater in the biosynthesis of chloroplast proteins than in the cytoplasmic ones. The magnitude of inhibition was dependent on exposure time and appeared to be related to sulphur uptake in the treated tissues.

INTRODUCTION

Sulphur dioxide affects various biochemical and physiological processes (1-5) as well as the ultrastructural organization of cellular organelles (1) prior to development of visual injury symptoms in needles of jack and lodgepole pines. The sensitivity to SO_2 of various metabolic processes depends on a number of factors, such as SO_2 concentration, duration of exposure, and the ability of the plant tissues to convert toxic species (HSO_3^-, $SO_3^=$) of the dissolved SO_2 to relatively less-toxic forms (6). Thus the factors that can limit the cellular concentration of SO_2--for example, stomatal opening and closing, enzymic and nonenzymic reactions catalyzing HSO_3^-, and $SO_3^=$ oxidation--can also determine the extent of SO_2 phytotoxicity (6).

Cellular and organelle membranes appear to be the sites most sensitive to SO_2 injury (1, 2). Since lipids and proteins are major components of biological membranes, we expected that SO_2 would have an effect on the biosynthesis of these two components. The exposure of pine needles to SO_2 has been shown to markedly inhibit the biosynthesis of lipids characteristic of chloroplasts and other cellular membranes (3). Decreased protein content has been reported in SO_2-treated plants (7-9). Such a decrease could occur due to either an inhibition in the protein biosynthesis or a stimulation in protein breakdown, or both. An increase in the amino acid content of jack pine needles after fumigation with SO_2 was suggested to be due mainly to increased breakdown of tissue proteins (4). At present, however, nothing is known about the effect of SO_2 on *de novo* biosynthesis of cellular proteins. In this study we therefore describe the influence of SO_2 fumigation on the *de novo* biosynthesis of chloroplast and cytoplasmic proteins of jack pine needles.

RESULTS AND DISCUSSION

Incorporation of $[U\text{-}^{14}C]$ leucine into needle proteins

Most of the radioactivity (90-95%) from $[U\text{-}^{14}C]$ leucine was incorporated into chloroplast and soluble cytoplasmic proteins. These two fractions incorporated about the same amount of radioactivity per gram dry weight of tissues: $74 \pm 4 \times 10^3$ cpm for the chloroplast fraction and $72 \pm 5 \times 10^3$ cpm for the soluble cytoplasmic fraction. The particulate fractions representing mitochondria and microsomes incorporated very little radioactivity into their proteins; therefore, only the soluble cytoplasmic and chloroplast fractions were used for measuring protein biosynthesis.

Treatment of the soluble cytoplasmic fraction with TCA precipitated only 15-20% of the total radioactivity, which suggests that a major portion of the label in the soluble cytoplasmic fraction was in the form of free leucine. This was further supported by the observation that 80-85% of the label present in the cytoplasmic fraction was lost upon dialysis.

Unlike in the soluble cytoplasmic fraction, all the radioactivity contained in the labelled chloroplast suspension was precipitated upon treatment with TCA, and the radioactivity was not lost upon washing the precipitated proteins with TCA and 80% acetone.

Centrifugation of the labelled chloroplasts on a discontinuous sucrose density gradient produced two distinct green bands: one at the interphases of sucrose concentrations of 30% and 40%, representing nonrefractile chloroplasts, and the other at the interphase between 40% and 50%, representing refractile chloroplasts (10). Analysis of the gradient fractions showed that the two chloroplast bands contained almost all of the total chlorophyll, protein, and radioactivity. Since most of the radioactivity was confined to the proteins representing the two chloroplast bands, the total chloroplast fraction was used in all subsequent experiments.

Specificity of amino acid incorporation into chloroplast proteins

The results in Table 1 show that the incorporation of alanine into chloroplast proteins was similar to that of leucine, but glutamic acid was poorly incorporated. These differences in incorporation could be attributed to carrier-mediated amino acid uptake across chloroplast membranes. It has been suggested that chloroplasts contain one specific carrier for alanine, leucine, glycine, and other aliphatic amino acids and another for serine, threonine, and methionine (11). Yet another carrier located on the inner membrane of the chloroplast envelope is involved in the transport of glutamic and aspartic acids (12, 13).

Incorporation of $\left[U\text{-}^{14}C\right]$ leucine into membrane-bound and soluble proteins of pine chloroplasts

The data in Table 2 show that a major portion (95%) of the radioactivity in the chloroplasts was associated with the membrane-bound protein fraction. The soluble protein fraction of the chloroplasts contained only a minor (5%) portion of the incorporation. Under *in vitro* conditions, isolated chloroplast suspensions have been shown to incorporate labelled amino acids into membrane-bound and soluble proteins (14, 17). The distribution of the label in the membrane-bound and soluble fractions was, however, different from the *in vivo* incorporation results of the pine needle chloroplast fractions reported here.

Effects of SO_2 fumigation on the biosynthesis of chloroplast and cytoplasmic proteins

Gaseous SO_2 is absorbed from the atmosphere through the stomata of plant leaves and is then dissolved within the moist tissues. It has been suggested that this dissolved gas is mainly responsible for SO_2 phytotoxicity (1, 2). The results in Table 3 show that exposure of pine seedlings to 0.34 ppm SO_2 for 24 hr inhibited chloroplast and cytoplasmic protein biosynthesis; this inhibition became more severe after 48 hr. Fumigation for 2 hr, however, produced little or no inhibition. No visual evidence of injury on pine seedlings was observed even after 48 hr exposure. At SO_2 exposures producing visual injuries, the severity of inhibition in protein biosynthesis was greatly increased. The results in Table 3 also show an increase in sulphur content of needles with increasing fumigation time, suggesting that inhibition of protein biosynthesis was related to increasing uptake of SO_2.

The inhibitory effect of SO_2 fumigation was more severe on the biosynthesis of chloroplast proteins than on that of cytoplasmic proteins. This effect could be due to the selective accumulation of SO_2 and its products in chloroplast membranes. It has been shown that fumigation of spinach leaves with $^{35}SO_2$ results in a preferential incorporation of ^{35}S in the thylakoid membranes (18). Among the protein fractions of the pine needle chloroplasts, the fumigation effect was considerably greater on the membrane-bound fraction than on the soluble protein fraction. A similar difference in SO_2 sensitivity has also been reported for the membrane-bound (mitochondrial) and soluble forms of glutamic-oxaloacetic transaminase in peas (19). These results clearly demonstrated that protein biosynthesis of different origins may have different sensitivities to SO_2. It is suggested that in pine needles SO_2 inhibited protein biosynthesis by acting either as a specific inactivator of the ribosomal system or as a nonspecific modifier or both. Fumigation with SO_2 has been shown to inhibit light-dependent activities of chloroplasts (20), and such changes are in turn expected to disrupt other metabolic processes.

The reduction in the protein content of SO_2-fumigated tissues (7-9) could, therefore, be attributed partly to inhibition in the *de novo* synthesis, as demonstrated in this investigation. We have found, however, that in addition to inhibiting *de novo* synthesis, SO_2 also caused increased breakdown of proteins. The results in Table 4 show that fumigation with SO_2 caused a decrease in the total protein content of both chloroplast and soluble cytoplasmic fractions. This decrease was more pronounced in the chloroplast proteins than in the cytoplasmic ones. This supports the suggestion that the increases found in the amino acid contents of SO_2-fumigated pine needles were due to breakdown of needle proteins (4). In *Lemna minor* stress has been shown to induce proteolysis and a decrease in the rate of protein synthesis, causing decreases in the activities of a number of enzymes (21). It is suggested that environmental stress such as SO_2 fumigation would bring about similar metabolic alterations.

The deleterious effects of SO_2 on the biosynthesis of proteins, especially of chloroplast membranes, would affect their availability as a structural component of new membranes and may also cause alteration in the existing membranes. The inhibition of polar lipids biosynthesis by SO_2 (3) would further aggravate the above process, since these lipids are the other structural components of the membrane. Alterations in the membrane lipids of the chloroplasts (galactolipids) affect membrane structure and the activity of Photosystem I (22). In pine, treatment of needles with aqueous SO_2 produced similar changes in the membrane structure (1), lipid composition (2), and Hill reaction activity of the chloroplasts (1).

EXPERIMENTAL

Chemicals. Omnifluor and l-$[U-^{14}C]$ amino acids (leucine, glutamic acid, and alanine) were purchased from New England Nuclear Corporation. Other chemicals were of commercially available highest grade.

Plant material and growth conditions. Jack pine (*Pinus banksiana* Lamb.) seedlings were grown as described earlier (1). Seedlings were watered as required and fertilized every week with Hoagland nutrient solution 2 diluted 1:1 with water (23). Mature needles from 4- to 5-month-old seedlings were used as experimental material.

Fumigation of seedlings with SO_2. Plant seedlings of uniform growth were carefully removed from the styroblock trays and transferred to cuvettes. Details of cuvette construction, seedling arrangement, and fumigation procedure were similar to those described earlier (3) except that the cuvettes used in the present study had an airflow rate of 100-120 l./min. The flow rates of SO_2 and air were measured and controlled with rotameters and valves before being mixed and introduced into the cuvettes. The concentration of SO_2 was monitored at the out port by a Phillips PW 9700 SO_2 analyzer and was maintained by a feedback controller. In each experiment 18-24 seedlings were equally divided into two sets and transferred to the cuvettes; one set received clean air, while the other set received 0.34 ppm SO_2.

Twenty-four hours prior to treatment, the cuvettes containing the plants were placed in a controlled environment chamber with a 19 500 lx light intensity (18 hr photoperiod) and 60-65% relative humidity. Temperatures in the growth chamber were 22° and 18° during the light and dark periods, respectively. The shoots were harvested after 2-, 24-, and 48-hr fumigation periods, and the excised fully mature needles (1-cm sections) were used for experimental purposes.

Metabolic experiments with $[U\text{-}^{14}C]$ *amino acids.* The excised needle tissues (2.0 g) were incubated in 20 ml distilled water containing 5 µCi 1-$[U\text{-}^{14}C]$ amino acid (sp. act. adjusted to 135 mCi/mmol with unlabelled amino acid) in a water bath shaker at 30° under 10 000 lx light intensity. Unless otherwise specified, $[U\text{-}^{14}C]$ leucine was routinely used as the labelled amino acid. Under these conditions the rate of amino acid incorporation into cellular proteins was linear up to 3 hr; therefore, a 3-hr period was routinely used in all experiments. After incubation each tissue sample was removed by filtration on a Miracloth, washed several times with cold distilled water, and then used for isolation of chloroplast and soluble cytoplasmic fractions.

Isolation of subcellular fractions. The labelled tissue was homogenized in 30 ml of 0.05 M K-Pi buffer (pH 7.0) containing 0.4 M sucrose, 1 mM dithioerythritol (DTE), 1 mM $MgCl_2$, and 1% PVP-10 for 30 sec by a Brinkman Polytron Homogenizer (Model PT-10) at a low speed. Care was taken to macerate all the needle tissues. The homogenate was filtered through two layers of moist Miracloth, and the filtrate was subjected to differential centrifugation: 1500 *g* for 5 min for chloroplasts and 15 000 *g* for 20 min

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for mitochondria. The sedimented particulate fractions were washed once with a suspending medium containing 0.05 M K-Pi buffer (pH 7.0), 0.4 M sucrose, 1 mM DTE, and 1 mM MgCl$_2$. The pellets were than suspended in a small volume of the above medium. The supernatant obtained after removing a 15 000-g pellet was centrifuged at 120 000 g for 60 min to obtain microsomal and soluble cytoplasmic fractions. All the above operations were carried out at 0-4°.

Density gradient centrifugation of the chloroplast fraction. Discontinuous sucrose gradients were prepared in centrifuge tubes by layering 2 ml each of 20%, 30%, 40%, 50%, and 55% sucrose solutions in a 0.05 M K-Pi buffer (pH 7.0) containing 1 mM DTE and 1 mM MgCl$_2$. A 0.5-ml aliquot of the chloroplast fraction was layered on the top of the gradient and then centrifuged at 0.4° for 20 min at 8 000 g in a swinging bucket rotor (Beckman SW41). Starting from the top, 1-ml fractions were collected and analyzed for chlorophyll and protein contents and ^{14}C incorporation.

Membrane-bound and soluble protein fractions of chloroplasts. To determine the incorporation of $\left[U\text{-}^{14}C\right]$ leucine in membrane-bound and soluble proteins, the chloroplast suspensions were ruptured by freezing and thawing followed by sonication for 1 min with a Biosonic-III Ultrasonicator at a medium setting. The suspension was then centrifuged at 120 000 g for 60 min. The resulting fractions were identified as soluble (supernatant) and membrane-bound (pellet). All the above operations were carried out at 0-4°.

Protein biosynthesis. A suitable aliquot of the isolated cellular fraction was mixed with TCA (final TCA concentration of 5%), and after 1-2 hr at 0°, the suspension was centrifuged. The precipitate was washed once with 1% cold TCA and twice with 80% cold Me_2CO. The washed precipitate was suspended in 0.5 N NaOH and was allowed to stand for 15 min in a water bath at 50°. Aliquots of the clear protein solutions were used for assaying the ^{14}C incorporation into proteins and the total protein content.

Radioactivity. Incorporation of ^{14}C in the proteins was assayed by mixing 0.1 to 0.5 ml of protein aliquot with 7 ml of scintillation fluid (0.4 g Omnifluor dissolved in a mixture of 30 ml absolute EtoH and 70 ml toluene).

Other estimations. Protein was measured according to ref. (24) and chlorophyll according to ref. (25). The tissue dry wt was measured by oven-drying the fresh needles at 80° for 24 hr. For sulphur analysis, the dried sample of needle tissues was ground to a fine powder, and a portion was used for oxygen flask combustion (26). Sulphur in the combusted sample was determined according to ref. (27).

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Table 1. Incorporation of $[U\text{-}^{14}C]$ alanine, $[U\text{-}^{14}C]$ leucine, and $[U\text{-}^{14}C]$ glutamic acid into proteins of jack pine chloroplasts

Amino acid	Incorporation (x 10^3 cpm/g dry wt)
Alanine	76.0
Leucine	75.0
Glutamic acid	23.7

Table 2. Distribution of [U-^{14}C] leucine incorporation into membrane-bound and soluble proteins of jack pine chloroplasts

Chloroplast fraction	Incorporation (x 10^3 cpm/g dry wt).
Total	71.3
Soluble	3.7
Membrane-bound	67.6

Table 3. Effects of SO_2 fumigation (0.34 ppm) on the biosynthesis of chloroplast and cytoplasmic proteins and on sulphur content of jack pine needles. *Sulphur content of the unfumigated needles = 0.9 ± 0.1 mg/g dry wt (± = S.D.)

Duration of SO_2 fumigation (hr)	Chloroplast proteins Total	Chloroplast proteins Membrane-bound	Chloroplast proteins Soluble	Cytoplasmic proteins	Sulphur* (mg/g dry wt)
	(% of control)	(% of control)	(% of control)	(% of control)	
2	97.0	96.7	97.0	100.0	0.9
24	72.2	71.6	85.4	79.6	1.3
48	54.9	54.0	69.5	70.4	2.4

Table 4. Effect of SO_2 fumigation (0.34 ppm) on total protein content of chloroplast and soluble cytoplasmic fractions of pine needles. Values of protein contents in the fractions isolated from control plants were: chloroplasts = 20.8 ± 1.0 mg/g dry wt; soluble cytoplasm = 24.6 ± 0.8 mg/g dry wt (± = S.D.)

Duration of SO_2 fumigation (hr)	Chloroplasts	Soluble cytoplasm
	(% of control)	
24	81.7	95.4
48	74.1	89.3

REFERENCES

1. Malhotra, S. S. (1976) *New Phytol.* **76**, 239.
2. Khan, A. A. and Malhotra, S. S. (1977) *Phytochemistry* **16**, 539.
3. Malhotra, S. S. and Khan, A. A. (1978) *Phytochemistry* **17**, 241.
4. Malhotra, S. S. and Sarkar, S. K. (1979) *Physiol. Plant.* **47**, 223.
5. Malhotra, S. S. and Khan, A. A. (1980) *Biochem. Physiol. Pflanz.* **175**, 228.
6. Malhotra, S. S. and Khan, A. A. (1982) in Air Pollution and Plant Life (Treshow, M., ed.). John Wiley and Sons, London [in press].
7. Fisher, K. (1971) *Mitt. Forstl. Bundes-Versuchsanst. Wien* **92**, 209.
8. Godzik, S. and Linskens, H. F. (1974) *Environ. Pollut.* **7**, 25.
9. Constantinidou, H. A. and Kozlowski, T. T. (1979) *Can. J. Bot.* **57**, 176.
10. Wagner, G. J. and Siegelman, H. W. (1975) *Science* **190**, 1298.
11. Nobel, P. S. and Cheung, Y. N. S. (1972) *Nature New Biol.* **237**, 207.
12. Heldt, H. W. and Saver, F. (1971) *Biochim. Biophys. Acta* **234**, 83.
13. Heldt, H. W. and Repley, L. (1971) in *Proceedings of the IInd International Congress on Photosynthesis Research* (G. Forti, M. Avron, and A. Melandri, eds.), p. 1345. Stressa, Junk, Hague.
14. Boardman, N. K., Franki, R. I. B. and Wildman, S. G. (1966) *J. Mol. Biol.* **14**, 470.
15. Chen, J. L. and Wildman, S. G. (1970) *Biochim. Biophys. Acta* **209**, 207.
16. Margulies, M. M. and Parenti, F. (1968) *Plant Physiol.* **43**, 504.
17. Harris, E. H., Preston, J. F. and Eisenstadt, J. M. (1973) *Biochemistry* **12**, 1227.

18. Ziegler, I. (1977) *Planta* **135**, 25.

19. Pahlich, E. (1975) *Atmos. Environ.* **9**, 261.

20. Shimazaki, K. and Sugahara, K. (1980) *Res. Rep. Natl. Inst. Environ. Stud. Japan* **11**, 79.

21. Cooke, R. J., Oliver, J. and Davies, D. D. (1979) *Plant Physiol.* **64**, 1109.

22. Shaw, A. B., Anderson, M. M. and McCarty, R. E. (1976) *Plant Physiol.* **57**, 724.

23. Hoagland, D. R. and Arnon, D. I. (1950) *Calif. Agric. Exp. Stn. Circ.* 347.

24. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.

25. Arnon, D. I. (1949) *Plant Physiol.* **24**, 1.

26. Chan, C. C. Y. (1975) *Anal. Lett.* **8**, 655.

27. Carson, J. A., Crepin, J. A. and Nemunis Siugzdines, P. (1972) *Can. J. Soil Sci.* **52**, 278.

SENSITIVITY TO SO_2 OF VARIOUS METABOLIC PROCESSES IN AN EPIPHYTIC LICHEN, *EVERNIA MESOMORPHA*

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Sensitivity to SO_2 of Various Metabolic Processes in an Epiphytic Lichen, *Evernia mesomorpha*

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Key Term Index: Sulphur dioxide effects; protein biosynthesis, photosynthetic CO_2-fixation; lipid biosynthesis; sulphur uptake; *Evernia mesomorpha*

Summary

In the epiphytic lichen *Evernia mesomorpha* Nyl., metabolic processes such as photosynthetic CO_2-fixation and protein and lipid biosyntheses were found to be very sensitive to SO_2. Exposure of lichens to 0.1 ppm of gaseous SO_2 for increasing durations produced a progressive reduction in these processes. Protein biosynthesis appeared to be the process most sensitive to SO_2. Fumigation of lichen tissues at 0.34 ppm of SO_2 for increasing durations caused an increased phytotoxic effect on all three metabolic processes. Such fumigations also inhibited acid phosphatase activity and caused an increase in the sulphur content of the tissues. During an SO_2-free period after the fumigations, these metabolic processes recovered partially or completely in lichens exposed to 0.1 ppm SO_2 but showed little or no recovery in lichens exposed to 0.34 ppm SO_2.

Introduction

Numerous lichen distribution and transplant studies around industrial developments or polluted areas have indicated a decline in the density and composition of species sensitive to SO_2, a major air pollutant in such areas. In view of this it was believed that SO_2 was responsible for the reduction in growth of sensitive lichens in such areas (SKYE 1968; GILBERT 1973; HAWKSWORTH 1973; FERRY and COPPINS 1979); however, little work has been done at the metabolic level to determine the effects and mechanism of SO_2 phytotoxicity in lichens.

RAO and LE BLANC (1965) showed that there was a breakdown of algal chlorophyll to phaeophytin after exposure of lichen thalli to lethal doses of SO_2 (5 ppm for 24 h). Such exposures also caused plasmolysis of cells and produced brown spots on the chloroplasts of phycobiont cells. In vascular plants, exposure to aqueous SO_2 caused

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Abbreviations: TAG, triacyl glycerol; PVP-10, polyvinyl pyrrolidone; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; DPDG, diphosphatidyl diglycerol chlorophyll breakdown (MALHOTRA 1977) and alteration in chloroplast ultrastructure (MALHOTRA 1976). Aqueous solutions of SO_2 have also been used to demonstrate the effects of SO_2 on lichen photosynthetic CO_2-fixation and respiration (HILL 1971; BADDELEY et al. 1972; PUCKETT et al. 1973); however, at present it is not known how the controlled exposure of lichens to low concentrations of gaseous SO_2 affects the above responses. In addition, little is known about the effect of gaseous SO_2 on the biosynthesis of proteins and lipids, two important membrane and cellular constituents.

On the basis of limited experimentation, O'HARE and WILLIAMS (1975) suggested that in certain lichens the degree of damage and sulphur accumulation was proportional to the rate of airflow. One may therefore expect that a low rate of airflow would limit the uptake of SO_2 from the atmosphere and that the small amounts of SO_2 thus absorbed would be quickly metabolized by lichen tissues into nontoxic forms. On the other hand, at the high flow rates that generally occur under field conditions, drying of lichen thalli would occur, thus limiting SO_2 absorption. In the field, the lichens absorb atmospheric SO_2 mainly during periods of high relative humidity. HAWKSWORTH (1973) suggested that a strong correlation could be made between lichen distribution and average SO_2 concentration during periods of high relative humidity. Moist atmospheric conditions may thus result in longer metabolically active periods during which lichens can absorb SO_2 and suffer more damage. In the field, however, lichens experience both wetting and drying cycles, and little study has been done on the effect SO_2 has on lichen metabolism under such conditions.

The present paper describes for the first time the protein and lipid biosyntheses and the effect of gaseous SO_2 on photosynthetic CO_2-fixation and the two biosyntheses in the epiphytic lichen *Evernia mesomorpha* Nyl. Recovery of various metabolic processes in an SO_2-free atmosphere was also studied in lichen tissues initially exposed to gaseous SO_2. Such information is lacking and must be obtained in order to explain the metabolic tolerance of lichen species to air pollution stress.

In this study we also attempted to simulate some important field environmental conditions in order to monitor the effects of different concentrations of SO_2 on a number of metabolic processes in *E. mesomorpha*. This species was selected because it appeared to be one of the species that was most sensitive to air pollution in the Athabasca oil sands area of northeastern Alberta (ADDISON and BAKER 1979).

Materials and Methods

Chemicals

$[U\text{-}^{14}C]$-leucine (spec. act. 355 mCi/mmol) and Omnifluor were purchased from New England Nuclear Corporation, and $[1\text{-}^{14}C]$-acetate (spec. act. 59 mCi/mmol) and $[^{14}C]$-sodium bicarbonate (adjusted to spec. act. 150 µCi/mmol) were purchased from Amersham Searle Corporation. Other reagents were of the highest quality commercially available.

Lichen collection

Branches laden with epiphytic lichens were collected from jack pine (*Pinus banksiana* Lamb.) stands in a relatively pollutant-free area near Fort MacKay in northeastern Alberta. When collected in the field the lichen samples were almost dry; they were brought to the laboratory in the same condition in polyethylene bags and were stored at -15°C in darkness until used. The lichen communities remained undisturbed during controlled fumigations in the laboratory. Only samples of *Evernia mesomorpha* Nyl. were harvested for analyses.

SO_2 fumigation

Small branches supporting *E. mesomorpha* were removed from cold storage and left overnight in a controlled environment chamber for preconditioning. The branches were divided into two groups: one group served as a control (untreated), and the other received SO_2 treatment. Both groups were moistened by a fine spray of distilled water at the start of the experiment. Branches supporting moistened lichen samples were mounted horizontally in cuvettes for both the control and the SO_2 samples. The cuvettes were placed in a controlled environment chamber and were maintained at 20°C and 4800-lux light intensity (16-h photoperiod). Humidified air (relative humidity 80-85%) was passed through the cuvettes at a flow rate of 10 l/min. The dimensions and operation of the cuvette have been described elsewhere (MALHOTRA and KHAN 1978). The low light intensity and reduced airflow rate were used to prevent rapid drying of the lichen samples. The concentration of SO_2 inside the cuvette was maintained by a feedback controller and was continuously monitored by a Phillips PW 9700 SO_2 analyser. In the experiments studying recovery of metabolic functions in an SO_2-free atmosphere, the supply of SO_2 to the cuvette was discontinued after a specified time, and the system was flushed with clean air. The lichens were then allowed to recover in clean air. The lichen samples were sprayed with distilled water at 08:00 and 16:00 during the treatment and recovery periods. Representative samples of the lichen were harvested and divided into about 1-cm segments before being assayed for various metabolic functions.

For all metabolic experiments, lichen material (0.1 g) was brought to full saturation by an initial incubation in distilled water (3-5 ml) for 10 min at 30°C.

Protein biosynthesis

Protein biosynthesis in the lichen tissues was measured by the incorporation of $[U\text{-}^{14}C]$-leucine into proteins. This was done by incubating the wet tissues in 5 ml of $[U\text{-}^{14}C]$-L-leucine (1.5 µCi) for 4 h at 30°C with constant shaking under a 9000-lux light intensity. The tissue was separated by filtration and was washed thoroughly with distilled water. The labeled tissue was then homogenized for 1 min in 10 ml of cold 0.1 M K-phosphate buffer (pH 7.0) using a Brinkman Polytron homogenizer (Model PT-10). Duplicate aliquots of each homogenate were mixed with trichloroacetic acid (final concentration 10%, v/v) to precipitate proteins. The suspension was mixed and allowed to stand in ice for several hours. It was then centrifuged at 4°C, and the residue was washed twice with cold 10% (w/v) trichloroacetic acid and once with cold 80% (v/v) acetone. The washed residue was then suspended in 0.5 N NaOH and left for 10 min in a hot-water bath (60-70°C) to solubilize the proteins. The insoluble material was removed by centrifugation, and an aliquot from the supernatant was assayed for $[^{14}C]$ incorporation into proteins.

Photosynthetic CO_2-fixation

For photosynthetic CO_2-fixation, the lichen tissue was incubated in 3 ml of $NaH^{14}CO_3$ (3.0 μCi) for 2 h at 9000-lux light intensity. The reaction was then stopped by adding 1 ml of formic acid (88%, v/v) and 16 ml of absolute ethanol. The contents were brought to a boil, cooled, and homogenized for 1 min with a Polytron homogenizer. The suspension was filtered through a Büchner funnel, and the residue was washed several times with hot 80% (v/v) ethanol. Aliquots of the filtrate were mixed with 0.1 ml of formic acid in scintillation counting vials and were flushed with a stream of N_2 in a hot-water bath to almost dryness. The residue was then dissolved in 0.1 ml of distilled water, and the fixed CO_2 was measured by counting $[^{14}C]$.

Lipid biosynthesis

Lipid biosynthesis was measured by assaying the incorporation of $[1\text{-}^{14}C]$-acetate into lipids. For this, the samples were incubated with 5 ml of $[1\text{-}^{14}C]$-acetate (10 μCi) under 9000-lux light intensity for 2 h at 30°C with gentle shaking. The tissue then was removed by filtration, washed several times with distilled water, and homogenized for 1 min in 20 ml of chloroform:methanol (2:1, v/v) using a Polytron homogenizer. The suspension was filtered through a Büchner funnel, and the residue was washed several times with chloroform:methanol (2:1, v/v). From the combined filtrate, total lipids were extracted by the method described by FOLCH et al. (1957).

Thin-layer chromatography of the labeled lipids was done using 0.5-mm silica gel H plates. Phospholipids were separated by developing the plates in lined tanks containing chloroform:methanol:acetic acid:water (100:25:10:4, v/v). For TAG, the plates were developed in a hexane:ethyl ether:formic acid (40:10:1, v/v) solvent system (MALHOTRA and KHAN 1978). Radioactive spots on the thin-layer chromatograms were identified by scanning the plates using a radioisotope scanner (Searle Actigraph III). These spots were matched with the standards, which were run beside or added to the sample. The various products in the chromatograms, including the standards, were visualized by exposure to iodine vapours. Silica gel from the radioactive regions was removed and counted for $[^{14}C]$.

Acid phosphatase (EC 3.1.3.2)

Lichen tissue (0.1 to 0.2 g) was incubated with 5 ml of distilled water for 10 min at 30°C. It was then mixed with 5 ml of a chilled solution of 0.1 M Tris-HCl buffer (pH 7.0) containing 0.5 mM dithioerythritol, 1 mM $MgCl_2$, and 2% (w/v) PVP-10. The contents were homogenized at 4°C with a Polytron homogenizer for 1 min, and the homogenate was filtered through two layers of cheesecloth. The filtrate was used immediately for assaying the enzyme activity (MALHOTRA and KHAN 1980).

Measurement of radioactivity

The incorporation of $[^{14}C]$ was measured by mixing the $[^{14}C]$ products in a scintillation vial with a counting fluid (0.4 g Omnifluor dissolved in a mixture of 30 ml of absolute ethanol and 70 ml of toluene). Counting was done with a Nuclear Chicago Isocap/300 liquid scintillation spectrometer.

Other estimations

The dry weight of a sample was measured by oven-drying the fresh tissues at 70°C for 24 h. For sulphur analysis, the dried sample was ground to a fine powder with a mortar and pestle, and a portion of the powder was used for oxygen flask combustion (CHAN 1975). Total sulphur content of the combusted sample was determined by the method of CARSON et al. (1972).

All experiments were repeated several times, and the trends were found to be highly reproducible. The data in the tables are taken from representative experiments. To compensate for biological variability on a day-to-day basis, an untreated control was analyzed simultaneously in each experiment and the results were expressed as a percent of the control. Mean values and the standard deviation for the controls (4-8 estimations) are provided in the tables.

Results and Discussion

De novo biosynthesis of proteins, lipids, and photosynthates in E. mesomorpha

Incubation of *E. mesomorpha* tissue segments in solutions of $[^{14}C]$ labeled compounds resulted in a *de novo* synthesis of proteins, lipids, and total photosynthates Table 1 (Table 1). The rates of these processes were linear with respect to reaction time under the assay conditions.

$[U\text{-}^{14}C]$-leucine was absorbed into the lichen tissues and incorporated into tissue proteins. About 10% of the absorbed label was incorporated into the proteins, while the remainder was in the form of free leucine.

The lichens incorporated $[1\text{-}^{14}C]$-acetate into various lipid fractions. Radio thin-layer chromatography showed that a major portion of the label was present in the TAG fraction. Among the phospholipid fractions, the incorporation was highest in PC, followed by PE, and then DPDG. Occasionally, an unidentified radioactive peak representing incorporation almost equal to the DPDG fraction was found in the hexane:ethyl ether:formic acid (40:10:1, v/v) solvent system. This fraction was located on the thin-layer plate just below the free fatty acid band. It has not been identified yet and is therefore not included in the table. Further analyses of these labeled lipid fractions after either alkaline hydrolysis or transmethylation showed that all the radioactivity was associated with the acyl moieties of the lipid fraction. These results indicate that *E. mesomorpha* contain a complement of the enzymes involved in the biosynthesis and acylation of fatty acyl moieties.

During photosynthesis, $[^{14}C]$-bicarbonate was also fixed by the lichen tissue into acid-stable and alcohol-soluble products. The relative distribution of the label into various photosynthates and the activity of various enzymes involved remains to be determined.

Effects of SO_2 on protein biosynthesis and photosynthetic CO_2-fixation

Increasing lengths of exposure of *E. mesomorpha* to 0.1 ppm SO_2 resulted ble 2 in progressive reduction of protein biosynthesis and photosynthetic CO_2-fixation (Table 2, expt. 1). Protein biosynthesis appeared to be more sensitive to SO_2 than did CO_2-fixation, especially after long exposures. It is important to note that even 2 days of exposure at such a low concentration resulted in about 15% inhibition in the rates of both processes. At a concentration of 0.34 ppm SO_2, the inhibitory effects on these processes occurred much faster and were more pronounced than at 0.1 ppm SO_2 (Table 2, expt. 2). Again, protein biosynthesis was inhibited more severely than photosynthetic CO_2-fixation. There was a considerable decline in the activity of both processes even after 2 h exposure time; 7 days of fumigation resulted in almost total inhibition of both processes.

The results in Table 2 also show the extent of recovery in the two metabolic processes when the fumigated lichens were transferred to an SO_2-free atmosphere. In lichens initially exposed to 0.1 ppm SO_2 a partial recovery was observed in both processes during the SO_2-free period. There was, however, no appreciable recovery of these processes in lichens initially exposed to 0.34 ppm SO_2 for 7 days (Table 2, expt. 2). This suggests that, under field conditions, if an exposure to low levels of SO_2 is followed by an SO_2-free period lichens may recover at least partially from the SO_2 stress, but due to their high metabolic sensitivity an exposure to high concentrations could cause irreversible injury. This would lead to severe reduction in lichen growth and their eventual death.

In vascular species, SO_2 has been reported to cause a decrease in protein content (MALHOTRA and KHAN 1982). Such an effect is possible due to either an inhibition in the *de novo* synthesis of the cellular proteins or an increase in the breakdown of existing proteins, or both. In *E. mesomorpha* exposed to SO_2, a marked reduction in the biosynthesis of proteins indicates that SO_2 inhibited at least the *de novo* synthesis of cellular proteins. Such a direct effect of SO_2 has not been previously demonstrated in either lichens or vascular species. Recently we also observed similar effects on vascular species (KHAN and MALHOTRA, unpublished results).

Similarly, there have been no reports of a direct effect of gaseous SO_2 on the photosynthetic CO_2-fixation in lichens. Most of the earlier work in this area was with lichens treated with solutions of HSO_3^-, SO_3^{2-}, and SO_2. For example, HILL,(1971) showed there was a reduction of photosynthetic CO_2-fixation in *Usnea subfloridana* treated with solutions of HSO_3^-. The effect was highly pronounced at pH 4.0 and lower. Similar reductions in photosynthetic CO_2-fixation have been reported by others (RICHARDSON and PUCKETT 1973; HALLGREN and HUSS 1975) following treatment of lichens with aqueous solutions of SO_2 or HSO_3^-.

Photosynthetic CO_2-fixation is catalyzed mostly by ribulose bisphosphate carboxylase (RuBPCase), and sulphite has been shown to inhibit the activity of partially purified (KHAN and MALHOTRA 1982) or impure (ZIEGLER 1972) preparations of this enzyme by competing for HCO_3^-/CO_2 binding sites. If metabolic processes in lichens are more sensitive to SO_2 or SO_3^{2-} than are those in vascular plants, one would expect that the RuBPCase activity of lichens would show lower *Ki* for SO_3^{2-}. ZIEGLER (1977), however, found that RuBPCase preparations isolated from *Pseudevernia furfuracea*, a lichen very sensitive to SO_2 (LE BLANC and RAO 1975), had higher *Ki* values for SO_3^{2-} than did a vascular plant. This led her to suggest that this lichen's sensitivity to SO_2 was not related to RuBPCase but was due to other physiological and morphological reasons. It is, however, important to stress that ZIEGLER (1977) used crude preparations of *P.*

furfuracea RuBPCase for studying the kinetics of SO_3^{2-} inhibition. The *Ki* values obtained by her may therefore have been influenced by the presence or absence of sulphite oxidase in the crude enzyme extracts. Recently we have shown that $Ki_{(SO_3^{2-})}$ can be substantially reduced for the partially purified low *Km* form of RuBPCase from *Pinus banksiana* (KHAN and MALHOTRA 1982).

Since low levels of SO_2 do inhibit CO_2-fixation in lichens, it is possible that SO_2 could combine with other cellular metabolites in lichens to form products more inhibitory to carboxylation than SO_3^{2-}. Alternatively, SO_2 could be interfering either with other enzymes of the Calvin cycle (if it is the only fixation mechanism) and their metabolic intermediates or with cellular membranes and associated physiological and enzymic reactions. The different degrees of SO_2 effects on protein biosynthesis and photosynthetic CO_2-fixation in *E. mesomorpha* clearly show that certain metabolic reactions within the same organism could be more sensitive than others. A similar differential sensitivity between nitrogen fixation and CO_2-fixation was shown in another lichen, *Stereocaulon paschale*, when it was treated with bisulphite solutions (HALLGREN and HUSS 1975).

Effects of SO_2 on lipid biosynthesis

ble 3

Long-term exposure of *E. mesomorpha* to 0.1 ppm SO_2 caused considerable reduction in the biosynthesis of all the glycerolipids (Table 3, expt. 1). This reduction became progressively more severe upon increasing the length of exposure to SO_2 from 15 to 29 days. This suggests that the inhibition and recovery of lipid biosynthesis were related to SO_2 toxicity. It appears that, similar to vascular species (MALHOTRA and KHAN 1978, 1980), lichens have a remarkable ability to recover from SO_2-related metabolic injury as long as the fumigation is with low levels for a limited time.

Exposure of *E. mesomorpha* to 0.34 ppm SO_2 produced marked reductions in the biosynthesis of all lipids (Table 3, expt. 2); the inhibition became severe after the first day. On Day 3 biosynthesis of all lipids was inhibited by more than 50%, and on Day 7 inhibition was 90-95%. In spite of such severe effects, there was some recovery in lipid biosynthetic capacity when the fumigated plants were transferred to an SO_2-free atmosphere for 7 days. The maximum recovery was in PC. These results suggest that various acylation reactions in *E. mesomorpha* have different sensitivities to SO_2. It is also possible that the biosynthesis of fatty acid chains, required for the acylation and synthesis of lipid species, was inhibited by SO_2. This would limit the availability of fatty acyl chains for acylation reactions in tissues and thus would inhibit the biosynthesis of various lipid species. Transmethylation or hydrolysis of the total lipids showed that reduced incorporation was due to a decrease in fatty acid synthesis. This indicates an inhibition of fatty acid synthetase activity.

Lipid biosynthesis in jack pine needles has also been shown to be markedly inhibited by low SO_2 concentrations that usually do not produce any visual symptoms of toxicity (MALHOTRA and KHAN 1978). Since lipids are essential constituents in the structure and function of cellular membranes, changes in their composition by SO_2 (KHAN and MALHOTRA 1977) were considered to be the primary reasons for adverse effects of SO_2 on pine needle cellular membranes (MALHOTRA 1976). Similar adverse effects could be anticipated in the membranes of *E. mesomorpha* as a result of SO_2 inhibition of lipid biosynthesis. Such alterations in the composition of lipids in the membranes would in turn affect important metabolic processes of the cellular membranes such as permeability, enzyme activity, and energy metabolism.

Effects of SO_2 on acid phosphatase

Treatment of *E. mesomorpha* with 0.34 ppm SO_2 for various lengths of time caused a decrease in lichen acid phosphatase activity, but the reduction was only 10% after 7 days of fumigation. The acid phosphatase activity of this lichen, therefore, appeared to be considerably less sensitive to SO_2 than were other cellular reactions. SCHMID and KREEB (1975) found that in another sensitive lichen species, *Parmelia physodes*, brief exposure to an unspecified low concentration of SO_2 neither affected the acid phosphatase activity nor caused any visual injury, but an unspecified high concentration produced marked reduction in the enzyme activity and discolouration of the thallus. There was also a wide scatter in the activity curve. In *E. mesomorpha*, a small decrease in the enzyme activity at a level that caused a marked reduction in other metabolic processes could be explained if SO_2 influenced the enzyme activity by differentially affecting the fungal and algal components of the lichen tissues. SO_2 may inhibit certain metabolic activity in one lichen component while stimulating activity in another component. In such a situation a composite assay involving the total tissues could be a misleading index of the tissue metabolic activity of individual components. If the metabolic processes in the algal cells are inhibited by SO_2 (which appeared to be the case, because photosynthetic CO_2-fixation was severely reduced), their sensitive symbiotic relationship with the fungal component would be disturbed.

Sulphur uptake of SO_2-fumigated lichens

Sulphur content of the lichen tissues increased appreciably upon fumigation at both levels of gaseous SO_2 (Table 4). The uptake was greater in lichens exposed to 0.34 ppm SO_2 for shorter periods than in lichens exposed to 0.1 ppm SO_2 for a longer period. This suggests that the metabolic turnover of the absorbed SO_2 was higher in the lichens exposed to a low SO_2 concentration for a longer duration. The fact that there was increased metabolic inhibition in treatments (Tables 2 and 3) involving exposure to high concentrations of SO_2 for shorter periods (Tables 2 and 3) supports this suggestion. It therefore appears that metabolic injuries are related to accumulation of absorbed SO_2 in the tissues. Table 4

Conclusions

The results of this study clearly indicate that 0.1 ppm of gaseous SO_2 can produce deleterious effects on lichen metabolism, but that such effects could be reversed in an SO_2-free atmosphere. The lichens appear to have an excellent ability to detoxify low levels of SO_2 (TURK et al. 1974). At 0.34 ppm of SO_2, however, the injury to various metabolic processes can be much more severe and not fully recoverable. Thus exposure of lichens to relatively high SO_2 concentrations in moist field conditions for even a short time can be expected to cause severe phytotoxic effects.

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References

ADDISON, P.A., and BAKER, J.: Ecological Benchmarking and Biomonitoring for Detection of Airborne Pollutant Effects on Vegetation and Soils. Interim Rep. LS 3.4, Alberta Oil Sands Environ. Res. Program, Edmonton, Alberta. 17 pp. (1979).

BADDELEY, M.S., FERRY, B.W., and FINEGAN, E.H.: The Effects of Sulphur Dioxide on Lichen Respiration. Lichenologist 5, 283-291 (1972).

CARSON, J.A., CREPIN, J.M., and NEMUNIS SIUGZDINES, P.: A Sulphate-sulphur Method used to Delineate the Sulphur Status of Soil. Can. J. Soil Sci. 52, 278-281 (1972).

CHAN, C.C.Y.: Determination of Total Sulphur in Vegetation by a Turbidmetric Method Following an Oxygen-flask Combustion. Anal. Lett. 8, 655-663 (1975).

FERRY, B.W., and COPPINS, B.J.: Lichen Transplant Experiments and Air Pollution Studies. Lichenologist 11, 63-73 (1979).

FOLCH, J., LEES, M., and SLOANE-STANLEY, G.H.: A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues. J. Biol. Chem. 226, 497-509 (1957).

GILBERT, O.L.: Lichens and Air Pollution. In: "The Lichens" (Edit. AHMADJIAN, V. and M.E. HALE) pp. 443-472. Academic Press, New York 1973.

HALLGREN, J.E., and HUSS, K.: Effects of SO_2 on Photosynthesis and Nitrogen Fixation. Physiol. Plant. 34, 171-176 (1975).

HAWKSWORTH, D.L.: Mapping Studies. In: "Air Pollution and Lichens" (Edit. FERRY, B.W., M.S. BADDELEY, and D.L. HAWKSWORTH) pp. 38-76. Athlone Press, London 1973.

HILL, D.J.: Experimental Study of the Effect of Sulphite on Lichens with Reference to Atmospheric Pollution. New Phytol. 70, 831-836 (1971).

KHAN, A.A., and MALHOTRA, S.S.: Effects of Aqueous SO_2 on Pine Needle Glycolipids. Phytochemistry 16, 539-543 (1977).

-- -- : Ribulose Bisphosphate Carboxylase and Glycollate Oxidase fróm Jack Pine: Effects of SO_2 Fumigation. Phytochemistry (1982) [In press].

Le BLANC, F. and D.N. RAO.: Effects of Air Pollutants on Lichens and Bryophytes. In: "Responses of Plants to Air Pollution" (Edit. MUDD, J.B., and T.T. KOZLOWSKI) pp. 237-268. Academic Press, New York 1975.

MALHOTRA, S.S.: Effects of SO_2 on Biochemical Activity and Ultrastructural Organization of Pine Needle Chloroplasts. New Phytol. 76, 239-245 (1976).

--: Effects of Aqueous SO_2 on Chlorophyll Destruction in *Pinus contorta*. New Phytol. 78, 101-109 (1977).

-- and KHAN, A.A.: Effects of Sulphur Dioxide Fumigation on Lipid Biosynthesis in Pine Needles. Phytochemistry 17, 241-244 (1978).

-- -- : Effects of Sulphur Dioxide and Other Pollutants on Acid Phosphatase Activity in Pine Seedlings. Biochem. Physiol. Pflanz. 175, 228-236 (1980).

-- -- : Biochemical and Physiological Impact of Major Pollutants. In: "Atmospheric Pollution and Plant Life" (Edit. TRESHAW, M.). John Wiley and Sons Ltd., London 1982 [In press].

O'HARE, G.P., and WILLIAMS, P.: Some Effects of Sulphur Dioxide Flow on Lichens. Lichenologist 7, 116-120 (1975).

PUCKETT, K.J., NIEBOER, E., FLORA, W.P., and RICHARDSON, D.H.S.: Sulphur dioxide: Its Effect on Photosynthetic $\left[^{14}C\right]$ Fixation in Lichens and Suggested Mechanisms of Phytotoxicity. New Phytol. 72, 141-154 (1973).

RAO, D.N., and LE BLANC, F.: Effects of Sulphur Dioxide on Lichen Alga with Special Reference to Chlorophyll. Bryologist 69, 69-75 (1965).

RICHARDSON, D.H.S., and PUCKETT, K.J.: Sulphur Dioxide and Photosynthesis in Lichens. In: "Air pollution and Lichens" (Edit. FERRY, B.W., M.S. BADDELEY, and D.L. HAWKSWORTH) pp. 283-298. Athlone Press, London 1973.

SCHMID, M.L., and KREEB, K.: Enzymatische Indikation gasgeschaedigter Flechten. Angew. Bot. 49, 141-154 (1975).

SKYE, E.: Lichens and Air Pollution. Acta Phytogeogr. Suec. 52, 1-123 (1968).

TURK, R., WIRTH, V., and LANGE, O.L.: CO_2-Gaswechsel-Untersuchungen zur SO_2-Resistenz von Flechten. Oecologia (Berlin) 15, 33-64 (1974).

ZIEGLER, I.: The Effect of $SO_3^{=}$ on the Activity of Ribulose-1, 5-diphosphate Carboxylase in Isolated Chloroplasts. Planta 103, 155-163 (1972).

--: Sulfite action on Ribulose Diphosphate Carboxylase in the Lichen *Pseudevernia furfuracea*. Oecologia (Berlin) 29, 63-66 (1977).

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Table 1: ***Biosynthesis of various metabolites in*** **E. mesomorpha**

Metabolites	Incorporation* (10^5 cpm/g dry wt of tissue)
Proteins	19.3 ± 2.6**
Lipids	
PC	11.7 ± 0.9
PE	7.1 ± 1.1
DPDG	5.1 ± 0.7
TAG	57.5 ± 6.5
Photosynthates	15.6 ± 1.2

* Labeled precursors as listed in materials and methods.

** Standard deviation.

Table 2: *Effects of SO_2 fumigation on protein biosynthesis and photosynthetic CO_2-fixation in* E. mesomorpha *and their recovery in an SO_2-free atmosphere*

Exposure time	Protein biosynthesis (% of control)	Photosynthetic CO_2-fixation (% of control)
Expt. 1 (0.1 ppm SO_2)		
2 days	85.5	85.2
15 days	35.2	65.3
29 days	11.6	32.6
29 days + 8 days SO_2-free	38.6	62.2
Expt. 2 (0.34 ppm SO_2)		
2 h	40.7	72.4
1 day	28.3	65.0
3 days	9.2	27.6
7 days	1.8	5.4
7 days + 7 days SO_2-free	3.6	18.5

Table 3: *Effects of SO_2 fumigation on lipid biosynthesis in* E. mesomorpha *and its recovery in an SO_2-free atmosphere*

Exposure time	Lipids			
	PC	PE	DPDG	TAG
	(% of control)			
Expt. 1 (0.1 ppm SO_2)				
15 days	92.5	84.4	74.6	77.0
29 days	54.0	55.2	72.7	43.7
29 days + 8 days SO_2-free	100.0	94.6	100.0	86.2
Expt. 2 (0.34 ppm SO_2)				
1 days	79.0	66.7	53.3	62.6
3 days	49.4	39.0	37.0	37.1
7 days	10.7	9.8	4.3	10.2
7 days + 7 days SO_2-free	51.2	37.2	25.0	38.8

Table 4: *Sulphur uptake of SO_2-fumigated* E. mesomorpha

Exposure time	Sulphur uptake (% of control*)
Expt. 1 (0.1 ppm SO_2)	
15 days	115.4
29 days	133.6
Expt. 2 (0.34 ppm SO_2)	
1 days	132.5
7 days	178.0

* Sulphur content of the control = 1.10 ± 0.05.

BIOCHEMICAL AND PHYSIOLOGICAL IMPACT OF MAJOR POLLUTANTS

by

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The harmful effects of air pollution on a variety of vegetation such as forest trees, agricultural crops, ornamental plants, and lichens are now well recognized. The major pollutants studied in this regard are sulphur dioxide, ozone, oxides of nitrogen, peroxyacetyl nitrate, and fluoride. These pollutants can have a deleterious effect on a variety of biochemical and physiological processes and on structural organization within the cells.

It is often assumed that there has been no injury to vegetation unless visible symptoms of phytotoxicity have developed. This can be misleading, however. In many controlled environment studies, air pollutants have been shown to reduce the growth and yield before any visible symptoms appeared. It is now commonly believed that the injury initially takes place at the biochemical level (interference with photosynthesis, respiration, lipid and protein biosynthesis, etc.) and progresses to the ultrastructural level (disorganization of cellular membranes), then cellular level (cell wall, mesophyll, and nuclei breakdown). Finally, visible symptoms develop (chlorosis and necrosis of foliar tissues).

Biochemical injury results when the concentration of the pollutant exceeds the capacity of the tissues to detoxify it through their normal metabolism. The subtle and varied nature of the biochemical and physiological effects produced by air pollutants suggests that reduction in plant growth and yield because of air pollution may be more widespread and serious than is generally suspected.

SULPHUR DIOXIDE

Stomatal response

Plants absorb sulphur dioxide (SO_2) mainly by gaseous diffusion through the stomata. Some uptake of SO_2 also occurs from moist cuticular surfaces but is of minor significance. The number of stomata and size of aperture play major roles in the uptake of SO_2, as do factors that can affect the turgidity of guard cells, such as humidity, wind velocity, light, and temperature. Thomas and Hill (1935) showed that absorption of SO_2 was correlated with humidity. The presence of SO_2 in the air has been shown to stimulate stomatal opening (Majernik and Mansfield, 1970, 1971; Mansfield and Majernik, 1970), or closing (Menser and Heggestad, 1966), both of which were regulated by relative humidity and the concentrations of SO_2 and CO_2 in the air (Majernik and Mansfield, 1972). Recently it was demonstrated in *Vicia faba* that low concentrations of SO_2 stimulated stomatal conductance within 15 minutes of exposure, which persisted for a several days (Black and Unsworth, 1980). This may have been due to extensive distruction of epidermal cells adjacent to the stomata (Black and Black, 1979a). High concentrations of SO_2, on the other hand, frequently caused severe ultracellular disorganization (Black and Black, 1979b).

The different tolerances of plant species to SO_2 under similar biophysical conditions suggest that fine differences in biochemical and physiological mechanisms operative in different plants could influence the sensitivity of a plant to SO_2. It has been suggested that SO_2 injury depends on the rate of SO_2 absorption (Thomas, 1951; Caput et al., 1978; Bressan et al., 1978). Recently, Furukawa et al. (1980a) found highly significant correlations between foliar injury and amount of SO_2 absorbed; plants sensitive to SO_2 absorbed greater amounts of gas than those resistant to it. In contrast, no correlation between sulphur uptake and foliar injury was observed in Agropyron smithii (Lauenroth et al., 1979). Resistance of Picea abies seedlings to SO_2 when stomata were open was attributed to cellular mechanisms of detoxifying SO_2 (Oku et al., 1980).

The effects of SO_2 on the biochemical and physiological processes directly related to stomatal response have been studied in detail recently. An increased concentration of carbon dioxide (CO_2) induces stomatal closure. Abscisic acid (ABA) is also known to produce a similar response and has the ability to control CO_2-induced stomatal closure (Raschke, 1975). Plant sensitivity to

SO_2 could therefore also be an indication of changes in the ABA levels of the fumigated foliage. Kondo and Sugahara (1978) measured the effect of 2 ppm SO_2 on the transpiration rates (a measure of stomatal behavior) of sensitive and resistant plants. They found a rapid decrease in the transpiration rate of SO_2- resistant plants and either a gradual decrease or an increase in transpiration rates of sensitive plants. Analysis of the ABA content of the foliage revealed that the resistant plants contained higher levels of ABA than the sensitive ones. When the ABA-treated *Raphanus sativus* was exposed to SO_2, the transpiration rate began to drop immediately, thereby making the plant much more resistant to SO_2 than it was originally. Later experiments by Kondo *et al*. (1980b) involving a number of SO_2- resistant plant species confirmed the relationship between ABA content and decreased transpiration rate following SO_2 fumigation.

Upon diffusion through the stomata, gaseous SO_2 dissolves in water on the moist cellular surfaces to form sulphite ($SO_3^=$), bisulphite (HSO_3^-), and other ionic species (depending on the pH of the surrounding cellular surfaces); in such transformation, cellular pH would also be influenced by the generation of protons. The influence of these species on the ABA related stomatal response was studied recently by Kondo *et al*. (1980b) using epidermal strips of *Vicia faba*. In the absence of ABA

$SO_3^=$ slightly stimulated stomatal opening, but in the presence of ABA, $SO_3^=$ produced no effect. No additional decrease in aperture size occurred by adding $SO_3^=$ in the presence of ABA concentrations that alone reduced aperture size. Changing the pH (between 4 and 7) similarly did not affect aperture size in the absence of ABA, but during its presence the stomatal aperture size was makedly reduced at pH 4. These results suggested that ABA-related stomatal closure as a result of SO_2 fumigation was an acidic effect on the surface or cytoplasm of guard cells. Other studies, however, have reported an increase in stomatal opening at low pH (Squire and Mansfield, 1972; Dittrich _et al._, 1979). Since in these studies the levels of ABA in the SO_2-fumigated plants were not examined before and after the fumigation (in either sensitive or resistant plant species), the role of ABA in regulating stomatal responses during fumigation remains speculative and contradictory. For example, the transpiration rate of _Zea mays_ leaves in response to SO_2 fumigation was similar to that of other resistant plant species, yet the ABA content of the leaves was the lowest of all the plant species examined. Even if the cellular content of ABA was a major factor in controlling the absorption of SO_2, the mechanisms involved in the regulation of ABA levels in guard and other subsidiary cells follwoing SO_2 fumigation remains to be determined.

It appears that stomatal regulation in plants not possessing the ABA-type mechanism occur by other biochemical and physiological means. Cellular increase of H^+ (normally brought about by SO_2) could cause leakage of K^+, Cl^-, and malate (Smith and Raven, 1979). In K^+ - regulated stomatal opening, anions such as malate and Cl^- play important roles (Raschke, 1979). Malate has been shown to be synthesized in the guard cells by carboxylation of phosphoenol pyruvate (PEP) (Outlaw and Kennedy, 1979). In _Zea mays_ and _Spinacea oleracea_, PEP carboxylase is inhibited by $SO_3^=$ (Ziegler, 1973; Mukerji and Yang, 1974), which in turn reduces malate synthesis and leads to changes in stomatal opening or plant sensitivity toward SO_2. Another biochemical control of stomatal response occurs through metabolic regulation of glycollate content (Zelitch, 1971). It has been shown that SO_2 inhibits glycollate oxidase activity in needles of _Pinus banksiana_ (Khan and Malhotra, 1981a); this enzyme is also inhibited by low concentrations of $SO_3^=$ (Zelitch 1957; Khan and Malhotra, 1981a).

Biochemical transformations of absorbed SO_2

The phytotoxic effects of SO_2 are greatly influenced by the ability of plant tissues to convert dissolved SO_2 into relatively non-toxic forms. Sulphite and HSO_3^- are the major chemical species formed upon dissolution of SO_2 in aqueous solutions; their respective concentrations depend on the pH of the medium (Puckett _et al_., 1973). Both $SO_3^=$ and HSO_3^- have been shown to be phytotoxic to many biochemical and physiological processes (Ziegler, 1975; Malhotra and Hocking, 1976). Plants can overcome these phytotoxic effects by converting $SO_3^=$ and HSO_3^- to less toxic forms. Oxidation of $SO_3^=$ to sulphate ($SO_4^=$) in plant cells can occur by both enzymic and non-enzymic mechanisms, and $SO_4^=$ thus accumulated is considerably less toxic than $SO_3^=$ (Thomas *et al.*, 1943). Plants exposed to SO_2 can accumulate sulphur compounds. Accumulation of large amounts of sulphur takes place at low SO_2 concentrations (Guderian, 1977), while at high SO_2 concentrations the accumulation is impaired due to collapse of stomatal regulation. In general, metabolically active young leaves accumulate more sulphur than older leaves (Guderian, 1977); however, *Agropyron smithii* accumulates less sulphur in young leaves than in the older ones (Lauenroth *et al.*, 1979). Experiments with isotopic SO_2 ($^{35}SO_2$) have shown that there are labelled $SO_3^=$ and $SO_4^=$ in the treated foliage (Garsed and Read, 1977a, b). Leaves of *Glycine max* exposed to $^{35}SO_2$ incorporated five times higher radioactivity in light than in dark (Garsed and Read, 1977a), the major product being $SO_4^=$ (Garsed and Read, 1977b). Studies on the residence time of $SO_3^=$ following SO_2 fumigation showed that *Glycine max* cultivars resistant to SO_2 converted $SO_3^=$ more rapidly to $SO_4^=$ than the sensitive cultivars (Miller and Xerikos, 1979). It appears, therefore, that the presence of $SO_3^=$-oxidizing mechanisms can influence plant resistance to SO_2.

Sulphite has been oxidized in light by isolated chloroplasts in a reaction induced by the electron transport system (McCord and Fridovich, 1969; Asada and Kiso, 1973; Libera et al., 1973; Khan and Malhotra, 1981b). Sulphate - oxidizing activities have also been reported in isolated mitochondria (Tager and Rautanen, 1956; Arrigoni, 1959; Ballantyne, 1977). Recently, Kondo et al. (1980a) separated various $SO_3^=$- oxidizing activities and characterized their natures. In a number of plants they found a cytochrome-c-linked $SO_3^=$- oxidizing substance that had a low molecular weight and was non-proteinaceous in nature. High molecular weight $SO_3^=$ oxidases, on the other hand, were not linked to cytochrome c. Oxidation of $SO_3^=$ can also be stimulated by cellular enzymes such as peroxidase, cytochrome oxidase, and ferredoxin-NADP reductase and by catalysts such as metals and ultraviolet light (Hallgren, 1978). Production of the superoxide radical (O_2^-) in the chloroplast during illumination also stimulates $SO_3^=$ oxidation. This is supported by the observation that in the presence of superoxide dismutase (SOD), photooxidation of $SO_3^=$ was inhibited (McCord and Fridovich, 1969; Asada and Kiso, 1973); however, free radical oxidation of $SO_3^=$ was accelerated by indoleacetic acid, tryptophan, and Mn^{+2} but not by SOD (Yang and Saleh, 1973). The superoxide radical formed during illumination has been found in vivo (Radmer and Kok, 1976) and in isolated chloroplasts (Asada and Kiso, 1973; Asada et al., 1974; Epel and Neumann, 1973); it could serve as a source of other active oxygens ($^1O_2$, $OH^\cdot$, and H_2O_2). In the presence of $SO_3^=$ and HSO_3^-, more O_2^- is formed by free radical chain oxidation. This process also generates $OH^\cdot$ and $SO_3^=$ radicals. Together these oxidizing radicals can affect a number of cellular mechanisms. In order to arrest uncontrolled production of such free radicals, plants contain natural scavengers (Asada, 1980) that protect them from injurious effects. For example, Tanaka and Sugahara (1980) found that leaves with a high content of SOD were resistant to SO_2 toxicity. Similarly, increased peroxidase activity that often results from fumigation of plants with SO_2(Keller, 1976; Keller _et al._, 1976; Horsman and Wellburn, 1975; Malhotra and Khan, 1981a) could be the result of either cellular activity to metabolize H_2O_2 produced by a free radical mechanism or the oxidation of absorbed SO_2. Ascorbic acid, another scavenger of free radicals, has also been reported to protect plants from SO_2 injury (Keller and Schwager, 1977; Grill _et al._, 1979a).

The $^{35}SO_2$ absorbed by plant leaves does not remain fixed at the site of absorption but has a substantial degree of mobility (Jensen and Kozlowski, 1975; Garsed and Read, 1977c); such translocations occur from leaves to roots, old leaves to young leaves, and roots to surrounding medium. Plants can, therefore, act as sinks for atmospheric SO_2.

Absorbed SO_2 can also be utilized by plants in the raductive sulphur cycle. Zeigler (1975) stated that this reduction seemed to involve reactions similar to SO_4 reduction. Since details of the reduction mechanisms have been well reported elsewhere (Schiff and Hodson, 1973; Hallgren, 1978), only recent work on this will be dealt with here. Sulphite reduction in plant leaves occurs mainly in the chloroplasts (Schiff and Hodson, 1973). Recently, it has been shown that in plants with C_4-type photosynthesis the assimilation of sulphur is initiated only in the bundle sheath chloroplasts; the mesophyll chloroplasts are inactive (Gerwick *et al*., 1980).

Sulphur dioxide can specifically inhibit the activity of adenosine phosphosulphate (APS) sulphotransferase in the leaves of *Phaseolus vulgaris* seedlings, but the inhibition can be reversed upon the removal of SO_2 (Wyss and Brunold, 1980). This is a key enzyme in the intermediary $SO_4^=$- reduction metabolism and is involved in in $SO_4^=$ transfer from APS to a carrier (Car-SH) to form a carrier-S-SO_3^- complex. Other sulphur compounds ($SO_4^=$, H_2S and cysteine) also affect the activity of this enzyme (Wyss and Brunold, 1979, 1980). It is possible that the high SO_2 concentration used in the above studies affected the enzyme due to excessive accumulation of $SO_4^=$ and other sulphur compounds. Recently it has been suggested that both $SO_3^=$ and $SO_4^=$ are transported to the inner chloroplast membranes by phosphate translocators (Hampp and Ziegler, 1977) and that light stimulates this process (Ziegler and Hampp, 1977). The light-activated uptake and reduction appears to be controlled by photosystem-dependent electron transport, since inhibition of the photoelectron transport system completely blocked both the uptake and reduction of $SO_4^=$ (Ziegler and Hampp, 1977). Sulphate reduction was dependent on ATP produced during photophosphorylation, because uncoupling of photophosphorylation inhibited $SO_4^=$ uptake and its reduction. Sulphite on the other hand, was metabolized directly, without any ATP requirement; in fact $SO_3^=$ uptake and reduction were stimulated by the uncoupler (Ziegler and Hampp, 1977). These results are of considerable significance in SO_2 metabolism, since $SO_3^=$ can be utilized directly in sulphur metabolism, especially in light of the inhibitory effects of SO_2 on chloroplast photoelectron transport and photophosphorylation systems (to be discussed later). In leaves of *Lemna minor*, however, it has been shown that absorbed SO_2 was assimilated only after oxidation to $SO_4^=$ (Brunold and Erismann, 1976). In support of direct reduction of SO_3, Ziegler (1977a) showed that sulphur from SO_2 and $SO_3^=$ was incorporated in the chloroplast thylakoids to a much greater extent than sulphur from $SO_4^=$. She suggested that $SO_3^=$ was either directly incorporated into the sulphonic groups of the sulphoquinovosyl moiety of the sulpholipids as reported by Benson (1963) or was taken up at the binding sites in the thylakoids (Schwenn *et al.*, 1976).

Accumulation of SH-containing amino acids following fumigation with SO_2 has been reported for a number of plant species (Ziegler, 1975; Grill et al, 1979b, 1980; Malhotra and Sarkar, 1979); similar accumulations have also been noticed upon continuous application of $SO_3^=$ to the cultures of Chlorella vulgaris (Soldatini et al, 1978). Photoreduction of SO_2 to H_2S and its subsequent release from plants was reported by Cormis (1968). Similar results were reports with $^{35}SO_2$ in Spinacea oleracea leaves and isolated chloroplasts (Silvius et al, 1976). Reduction of SO_2 to H_2S could be catalyzed by sulphite reductase, found in many plants (Schiff and Hodson, 1973). It is interesting to note that short fumigation of Spinacea oleracea plants with SO_2 caused a marked increase in free and masked thiol in the chloroplasts (Miszalski and Ziegler, 1979). Such an increase in the concentration of SH compounds can influence the natural balance of SH/SS in the cells. Plants exposed to injurious concentrations of SO_2 emit considerable amounts of H_2S; a positive correlation between such emissions and SO_2 resistance was shown in a recent report by Sekiya et al. (1980).

Photosynthesis

The response of photosynthetic processes to SO_2 depends on the duration of exposure and concentration of the SO_2. Short exposure to low SO_2 concentrations generally stimulate photosynthesis in a number of plants. In most cases, however, high doses of SO_2

or continued exposures at even low concentrations are very injurious to photosynthesis. The sensitivity of plants to SO_2 at low concentrations can, however, be influenced by environmental factors such as wind speed, light, and humidity. Ashenden and Mansfield (1977) showed that 0.11 ppm SO_2 significantly reduced the growth of Lolium perenne only at high wind speed; the lack of effect at lower wind speed was due to increased boundary layer resistance. In Vicia faba, a decrease in net photosynthesis occurred at SO_2 concentrations exceeding 0.035 ppm, and the decrease was influenced by boundary layer resistance and light itensity (Black and Unsworth, 1979a and b). Hallgren (1978) has reviewed physiological conditions that influence the SO_2 effect on the photosynthetic response of intact plant leaves.

A rapid decrease in net photosynthesis in leaves of Helianthus annuus upon exposure to 1.5 ppm SO_2 led Furukawa et al. (1980b) to suggest that the chloroplasts were the primary site of attack. Biochemical reactions associated with photosynthesis have been studied in order to ascertain more specifically the sites and mechanisms of SO_2 action. Ziegler (1972) observed that $SO_3^=$ inhibited ribulose diphosphate (RuDP) carboxylase in Spinacea oleracea chloroplasts. The kinetics ot $SO_3^=$ inhibition indicated competition between HCO_3^- and $SO_3^=$ at the CO_2-binding sites of the enzyme, which suggests that the concentration of CO_2 (or HCO_3^-) at the site of carboxylation influences the degree of $SO_3^=$ inhibition. A similar type of competitive inhibition by

$SO_3^{=}$ with respect to HCO_3^- has been observed with isolated preparations of RuDP carboxylase from a lichen, *Pseudevernia furfuracea* (Ziegler, 1977b). Recently, however, Gezeilus and Hallgren (1980) showed that the $SO_3^{=}$ inhbition of RuDP carboxylase from *Pinus silvestris* and *Spinacea oleracea* was non-competitive with respect to HCO_3^-, and the nature of the inhibition was not affected by the presence of $SO_3^{=}$ during the activation. It is interesting to note that the enzyme from *Pinus silvestris* was inhibited similary by 10 mM $SO_3^{=}$ or $SO_4^{=}$. Since $SO_4^{=}$ inhibits *Spinacea oleracea* RuDP carboxylase non-competitively with respect to HCO_3^- (Trown, 1965), the possible presence of $SO_3^{=}$-oxidizing systems in crude enzyme extracts of *Pinus silvestris* cannot be overruled. Gezeilus and Hallgren (1980) have minimized such an interference by assaying in N_2 atmosphere. Using purified preparations of RuDP carboxylase from *Pinus banksiana*, Khan and Malhotra (1981a) found a caompetitive type of inhibition by $SO_3^{=}$, with a $Ki(SO_3^{=})$ value of 5 mM; the enzyme was also inhibited by $SO_4^{=}$, but to a much lesser extent than by $SO_3^{=}$. It must be pointed out that crude extracts and isolated chloroplasts of *Pinus banksiana* needles contain very active $SO_3^{=}$-oxidizing systems (Khan and Malhotra, 1981b). It is therefore necessary that purified preparations of RuDP carboxylase be used for assessing the nature of $SO_3^{=}$ inhibition. Reduction in the activity of RuDP carboxylase was also observed in *Alnus crispa* and *Betula papyrifera* seedlings upon fumigation with 0.34 and 0.51 ppm

SO_2 (Khan and Malhotra, 1981c). Reduction in the activity of RuDP carboxylase upon exposure to gaseous SO_2 has also been reported in other plant leaves (Horsman and Wellburn, 1975; Miszalski and Ziegler, 1980).

Sulphite also affects phosphoenolpyruvate (PEP) carboxylase (Ziegler, 1973a; Mukerji and Yang, 1974) which is involved in C_4 pathway of photosynthesis. In Zea mays (Ziegler, 1973a), the $SO_3^=$ inhibition was competitive with respect to HCO_3^-, similar to inhibition of RuDP carboxylase in Spinacea oleracea (Ziegler, 1972); however, $Ki(SO_3^=)$ of 27 mM with respect to PEP carboxylase was much higher than that of RuDP carboxylase (3 mM). Since PEP carboxylase has much higher affinity for CO_2 [$Km(HCO_3^-)$ of 0.5 mM] (Ziegler, 1973a), the replacement of CO_2 by $SO_3^=$ at the carboxylation site would be more difficult in the PEP carboxylase catalyzed reaction than in the RuDP carboxylase reaction. The effect of $SO_3^=$ on PEP carboxylase from Spinacea oleracea (Mukerji and Yang, 1974) appeared to be different from that of the Zea mays enzyme. The Spinacea oleracea enzyme was stimulated by $SO_3^=$ at a low concentration (0.5 mM) and was inhibited at a high concentration (5 mM). Sulphite inhibited the enzyme activity, and the inhibition was of a mixed type with respect to HCO_3^-. Kinetic analysis of the Spinacea oleracea enzyme suggests that $SO_3^=$ affected PEP carboxylase activity, not by interfering with the substrate binding sites, but by unspecific binding with the enzyme protein (Mukerji and Yang, 1974). Since $SO_3^=$ has been shown to bind rapidly with PEP in vitro (Lehmann and

Benson, 1964), such a reaction during PEP carboxylase assay would limit the availability of PEP for carboxylation. In the presence of PEP carboxylase, however, a $SO_3^{=}$ reaction with PEP did not appear to occur because $SO_3^{=}$ either had no effect or acted non-competitively with respect to PEP (Ziegler, 1973a; Mukerji and Yang, 1974). Bisulphite compounds have also been shown to inhibit PEP carboxylase reaction (Osmond and Avadhani, 1970; Mukerji and Yang, 1974). The extent to which PEP carboxylase activity is influenced by gaseous SO_2 has not been determined.

In addition to influencing the carboxylation reactions, SO_2 can affect photosynthesis by attacking photosynthetic electron transport and photophosphorylation reactions. Chloroplasts isolated from needles of Pinus contorta treated with varying concentrations of aqueous SO_2 showed that at a low concentration (50 ppm), SO_2 stimulated Hill reaction activity, but this activity was completely inhibited at high concentrations (500-1000 ppm) (Malhotra, 1976). The chloroplasts isolated from old tissues were more sensitive to SO_2 than were those from young, actively growing tissues. A decrease in the Hill reaction activity was accompanied by swelling and disintegration of chloroplast membranes. Such alterations in the membranes can cause disorganization of the two photosystems. Photosystems I and II are both localized in the membranes of chloroplasts and have been separated by density gradient centrifugation of digitonin-treated chloroplasts (Boardman, 1968).

Recently Shimazaki and Sugahara (1980a,b) studied in detail the effect of gaseous SO_2 on chloroplast photosystems in Spinacea oleracea. Fumigation with SO_2 at 1 and 2 ppm for 1 hour produced no effect on 2,6-dichloroindophenol (DCIP) photoreduction (Hill reaction); however, there was rapid inhibition following longer exposures. Shimazaki and Sugahara investigated the site of SO_2 attack in the electron transport systems by studying both photosystems.

Electron transport of both the whole chain and Photosystem II was inhibited to the same magnitude by SO_2, but SO_2 did not inhibit electron flow from reduced DCIP-NADP under uncoupled conditions, which suggests that the site of SO_2 action in the photosystems was associated with Photosystem II and not Photosystem I. A similar effect of SO_2 was observed in Photosystems of Lactuca sativa chloroplasts (Shimazaki and Sugahara, 1980b). The work with chloroplasts isolated from SO_2-fumigated leaves of Lactuca sativa (Shimazaki and Sugahara, 1980b) demonstrated that the site of SO_2 action was located closer to the oxidizing side rather than the reducing side of Photosystem II. This was supported by the observation that the addition of an artifical electron donor diphenylcarbazide (DCP) did not change the rate of DCIP reduction in Photosystem II. The work of Shimazaki and Sugahara (1980b) also suggests that

SO_2 did not inactivate the electron flow from the reductant to the primary electron acceptor (Q) of Photosystem II. Time-course analysis of fluorescence intensity in SO_2-treated plants indicated that SO_2 inhibited the accumulation of reduced Q. Furthermore, the addition of DCMU, an inhibitor acting on the reducing side of Photosystem II (Bishop, 1958), caused a rapid increase in fluorescence in SO_2-inhibited chloroplasts, which suggests that Q was in the oxidized state. This could happen because of SO_2 inactivation of either the primary electron donor or the reaction centre itself in the electron transport chain.

The above inhibitory effects of gaseous SO_2 on the photosystems are different from those reported from earlier studies in which treatment of isolated chloroplasts with solutions of $SO_3^=$ either produced no overall effect on electron transfer (Asada, et al., 1965) or stimulated a non cyclic type of electron transfer (Libera et al., 1973). The effect of gaseous SO_2 seems to be specific and not associated with the acidity-related decrease in Photosystem II activity since a decrease in Photosystem II activity due to low PH could be restored by adding electron donors of Photosystem II but not in chloroplasts from SO_2 treated plants (Shimazaki and Sugahara, 1980b). Inhibition of Photosystem II activity by exposure to SO_2 was accompanied by a similar inhibition in non-cyclic photophosphorylation, but not cyclic photophosphorylation (Shimazaki and Sugahara, 1980a).

On the other hand, treatment of isolated chloroplasts with solutions of $SO_3^=$, HSO_3^-, and SO_2 inhibited both cyclic and non-cyclic photophosphorylations (Asada *et al*, 1965; Libera *et al*., 1973; Silvius *et al*, 1975).

The differences between the *in vivo* effects of SO_2 on both photoelectron transport and phosphorylations and the *in vitro* effects of treatment of isolated chloroplasts with aqueous SO_2 (HSO_3^-, $SO_3^=$, and SO_2) are difficult to reconcile. Shimazaki and Sugahara (1980a) have attributed this to production of O_2^- and other radicals during photooxidation of $SO_3^=$. Since $SO_4^=$ is formed by the oxidation of $SO_3^=$, it is possible that the effects observed *in vitro* are the effects of free radicals and $SO_4^=$. It has been shown that $SO_4^=$ irreversibly inhibits both cyclic and non-cyclic photophosphorylations (Ryrie and Jagendorf, 1971).

Pigments

Since chlorophyll and other plant pigments are necessary in harnessing light energy by Photosystems I and II, the effect of SO_2 on these pigments would greatly influence the photosynthetic ability of plants. Rao and LeBlanc,(1965) found that destruction of chlorophyll occurred in lichens following exposure to large doses (5 ppm for 24 hours) of gaseous SO_2. At this high concentration, chlorophyll molecules were degraded to phaeophytin and Mg^{2+}. A similar conversion of chlorophyll to phaeophytin can occur with acids or acidic substances. In this process Mg^{2+} in the chlorophyll molecule is replaced with two atoms of hydrogen, thereby changing the light spectrum characteristic of the chlorophyll molecules. A decrease in chlorophyll content has been often suggested as an indicator of air pollution (mainly SO_2) injury. In sensitive lichens, chronic exposure to even a low concentration (0.01 ppm) of SO_2 resulted in a loss of chlorophyll (Gilbert, 1968). In *Evernia mesomorpha*, control fumigation with low level of SO_2 caused a gradual decline in the chlorophyll content, which was accompanied by a decrease in photosynthesis; the effects on both chlorophyll destruction and photosynthesis became more rapid and pronounced at 0.34 ppm SO_2 concentration (Malhotra and Khan, 1981b).

Sulphur dioxide can influence plant chlorophyll by various mechanisms. Malhotra (1977) showed that treatment of *Pinus contorta* needles with aqueous SO_2 markedly affected the chlorophyll content of the foliage and that this was not due to increased acidity alone. Since, *in vivo*, chlorophyll is stabilized by its organization as a complex with proteins, it is possible that SO_2 first attacks this complex before the actual breakdown of chlorophyll occurs. In *Pinus*, low aqueous SO_2 concentrations had very little effect on either chlorophyll *a* or *b*, but at high concentration Chlorophyll *a* appeared to be much more sensitive to aqueous SO_2 than Chlorophyll *b*. Increased destruction of Chlorophyll *a* was accompanied by a parallel increase in Phaeophytin *a* and loss of Mg^{2+} from the tissues. Since the destruction of chlorophyll in pine needless was markedly greater in the presence of aqueous SO_2 than in the presence of acid solutions of the same pH, it was suggested that SO_2 destroyed chlorophyll as a result of its strong redox properties. In needles of *Pinus contorta*, a decline in Chlorophyll *b* content in the presence of SO_2 did not produce a corresponding increase in Phaeophytin *b* which suggests a different mechanism in Chlorophyll *b* breakdown. In fact, an increase in Chlorophyllide *b* content following SO_2 treatment suggested that Chlorophyll *b* breakdown was probably a result of splitting of the phytol chain by chlorophyllase. The maximum increase in tissue Chlorophyllide *b* content was observed at 50 ppm aqueous SO_2 concentration, the same concentration that produced the maximum stimulation in chlorophyllase activity.

Rapid *in vitro* chlorophyll destruction can also be caused by free radicals produced during the oxidation of HSO_3^--catalyzed decomposition of linoleic acid hydroperoxide (Peiser and Yang, 1977, 1978). Recently Shimazaki *et al*. (1980) presented evidence that SO_2 fumigation of plant leaves increases the formation of O_2^- in chloroplasts which in turn destroys chlorophylls. Superoxide radical has been shown to influence chlorophyll at very low concentrations (10^{-8} to 10^{-7}M) (Asada *et al*., 1977). In *Spinacea oleracea* leaves, gaseous SO_2 destroyed Chlorophyll *a* more rapidly than Chlorophyll *b*, but the loss of Chlorophyll *a* was not accompanied by a corresponding increase in

Respiration

Photorespiration has been found to be inhibited by SO_2 (Ziegler, 1975). Exposure of *Lolium perenne* to SO_2 (0.15 ppm) caused an inhibition in glycine and serine synthesis, even though CO_2 fixation was stimulated; this suggests inhibition of photorespiration (Koziol and Cowling, 1978). A similar reduction in these amino acids following exposure to SO_2 was reported by Tanaka *et al.*, (1972b). Glycollate oxidase, an important enzyme for the synthesis of glycine and serine, was inhibited by low concentrations of $SO_3^{=}$ *in vitro* (Ze litch, 1957; Paul and Bassham, 1978; Khan and Malhotra, 1981a) and by gaseous SO_2 (Khan and Malhotra, 1981a). In leaves of *Nicotiana tabacum*, however, exposure to a high SO_2 concentration (1.3 ppm for 18 hours) induced an increased synthesis of glycollate oxidase (Soldatini and Ziegler, 1979). It has been suggested that a decrease in phtorespiration as a result of SO_2 or $SO_3^{=}$ exposure is due to the formation of glyoxylate bisulphite, which is a potent inhibitor of glycollate oxidase (Zelitch, 1957). Glyoxylate bisulphite was found to accumulate in the leaves of *Oryza sativa* plants exposed to high concentrations of SO_2 (Tanaka *et al.*,1972a). Similarly, *Pisum sativum* exposed to high SO_2 concentration produced toxic bisulphite compounds of glyceraldelyde, α-ketoglutarate, pyruvate, and oxaloacetate (Jiracek *et al.*, 1972). The high concentration of SO_2 used in these studies casts doubts on the formation of such compounds in plants exposed to the more-common low levels of SO_2 experienced under field conditions.

Unlike photorespiration, dark respiration either showed no response or was stimulated by SO_2 (Black and Unsworth, 1979b; Furukawa *et al.*, 1980b). The significance of these results at the biochemical level was not ascertained. The *in vitro* addition of $SO_3^=$ to plant mitochondrial preparations, however, inhibited the formation of ATP (Ballantyne, 1973). It is possible that due to its strong reducing nature, $SO_3^=$ caused reduction of electron transport components and uncoupling, which resulted in subsequent decreased ATP synthesis.

Sulphur dioxide and its dissolved reactive species can affect a variety of other cellular metabolites and enzyme systems through either non specific or specific mechanisms. At high concentrations, $SO_3^=$ reacts with a number of metabolites (Mudd, 1975a; Petering and Shih, 1975; Malhotra and Hocking, 1976), but at low concentrations, generally present under field conditions, such reactions will be of rare occurrence. The following sections discuss the effects of low and medium concentrations of SO_2 on enzymes and cellular metabolites.

Amino acids and proteins

In a number of plant species free amino acid content increases following SO_2 fumigation (Arndt, 1970; Jäger and Grill, 1975; Malhotra and Sarkar, 1979), however in sulphur-deficient

Lolium perenne, SO_2 fumigation caused a decrease in total amino acids and amines, asparagine, and glutamine (Cowling and Bristow, 1979). In general, SO_2 fumigation results in an increase specifically in sulphur-containing amino acids (Ziegler, 1975).

Increased contents of glycine, alanine, threonine, lysine and methionine in needles of *Pinus banksiana* treated with SO_2 were thought to be due mainly to increased breakdown of needle proteins (Malhotra and Sarkar, 1979). Further work showed that SO_2 decreased the soluble cytoplasmic and chloroplast protein contents of the needles and that the decrease was higher in the chloroplast than in the soluble cytoplasmic fraction (Khan and Malhotra, 1981d). A decrease in the total protein content upon SO_2 fumigation has been reported for a number of plants (Fischer, 1971; Godzik and Linskens, 1974; Constantinidou and Kozlowski, 1979). Such a decrease could be attributed to breakdown of the existing proteins and to reduced *de novo* synthesis. Exposure of an epiphtic lichen, *Evernia mesomorpha*, to 0.1 ppm SO_2 for 2 days resulted in a considerable reduction in protein biosynthesis, and this effect became more severe after longer exposures. Fumigation for 3 days at 0.34 ppm SO_2 caused a very pronounced inhibition that was irreversible in clean air (Malhotra and Khan, 1981b). Similarly, SO_2 (0.34 ppm) markedly inhibited *de novo* biosynthesis of both cytoplasmic and chloroplast proteins in *Pinus banksiana*, and the effect was greater on the chloroplast proteins (Khan and Malhotra, 198~~1d~~2e). These results indicate that membrane-associated processes are more sensitive to SO_2 than the cytoplasmic processes.

In Pisum sativum, SO_2 fumigation at 0.3 ppm concentration for 18 days resulted in a marked increase in free and bound polyamines such as putrescine and supermidine (Priebe et al., 1978). Polyamines which are metabolic products derived from amino acids, play important roles in nucleic acid metabolism and in the regulation of cellular pH (Cohen, 1971). The increased formation of polyamines in Pisum sativum leaves upon exposure to SO_2 was accompanied by a marked increase in the contents of arginine and ornithine, the precursors of putrescine and supermidine (Preibe et al., 1978). The arginine content of Pinus banksiana needles also increased upon fumigation with SO_2 (Malhotra and Sarkar, 1979), however, its relevance to polyamine synthesis was not studied. The accumulation of these metabolic products following SO_2 fumigation led Priebe et al. (1978) to suggest that increased polyamine synthesis was a regulatory process for binding excessive H^+ that was produced in the tissues as a result of SO_2 absorption. It has been suggested that polyamines, whose basicity is comparable to NaOH, could form polyvalent cations by binding H^+ and thus would act as buffering compounds in the cells (Priebe et al., 1978). In seedlings of Hordeum vulgare, acidic conditions were shown to activate the enzymes arginine decarboxylase and N-carbamylputrescine amidohydrolase, which are involved in the synthesis of putrescine (Smith and Sinclair, 1967).

Several enzymes involved in amino acid metabolism have also been shown to be affected by SO_2. In Pisum sativum, fumigation with SO_2 affected glutamate dehydrogenase activity by stimulating reductive amination on one hand and inhibiting oxidative deamination on the other (Pahlich et al., 1972). These effects were attributed to changes in isoenzyme pattern (Pahlich et al, 1972) and to enzyme kinetics (Pahlich, 1971). Fumigation of Pisum sativum seedlings with SO_2 inhibited the mitochondrial glutamate oxaloacetate transaminase; the cytoplasmic enzyme showed no effect (Pahlich, 1973). The inhibition of the mitochondrial enzyme by $SO_3^{=}/HSO_3^{-}$ was of a mixed type. On the other hand, the transaminase(s) activity (glutamate-oxaloacetate and glutamate-pyruvate) of Pisum sativum seedlings was slightly stimulated by 0.2 to 2 ppm SO_2 fumigation (Horsman and Wellburn, 1975). Rabe and Kreeb (1980), however, showed that transaminases (alanine and aspartate) were stimulated only by low SO_2 concentrations; at high concentrations they were inhibited.

Lipids and fatty acids

Lipids are important constituents of biological membranes. In chloroplasts, glycerolipids constitute about 50% by weight of thylakoid membranes (James and Nichols, 1966). A major portion of these glycerolipids is present as monogalactosyl diglyceride, digalactosyl diglyceride, and sulphoquinovosyl diglyceride. In Pinus contorta, SO_2 caused a marked reduction in the concentration and composition of these glycolipids (Khan and Malhotra, 1977) in both young and fully developed needles. Since galactolipids have been shown to be involved in the structure and function of chloroplasts (Shaw *et al.*, 1976), it was suggested that structural alterations in *Pinus contorta* chloroplasts (Malhotra, 1976) were due to changes in galactolipid concentration and composition (Khan and Malhotra, 1977). Isolated chloroplast preparations of *Pinus banksiana* needles contained enzyme systems responsible for the biosynthesis of these membrane lipids (Khan and Malhotra, 1978).

Lipid biosynthesis in an epiphytic lichen, *Evernia mesomorpha*, has been shown to be affected by SO_2 (Malhotra and Khan, 1981b). Inhibition caused by low-level SO_2 exposures (0.1 ppm) was completely recovered 8 days after termination of fumigation. At 0.34 ppm SO_2, biosynthesis was inhibited even after 1 day of fumigation, and on Day 3 this inhibition became very severe; there was very little recovery in biosynthetic activity upon removal of SO_2.

Decreases in the lipid content following SO_2 exposure may be brought about by either reduced synthesis, increased lipase activity, peroxidation of fatty acid chains, or a combination of the above. Malhotra and Khan (1978) showed that exposure of *Pinus banksiana* and *Pinus contorta* seedlings to SO_2 caused a marked inhibition in the *de novo* synthesis of phospho-, glyco-, and neutral lipids. The magnitude of inhibition was dependent on the concentration and duration of exposure to SO_2. The effect was, however, transitory in nature, since the inhibition was partially or completely reversed upon removal of plants from the SO_2 atmosphere. Since no accumulation of free fatty acids occurred upon SO_2 treatment, it was suggested that SO_2 did not cause stimulation of lipolytic activity but inhibited the synthetic and acylation processes.

Treatment of Pinus banksiana needles with SO_2 produced a marked reduction in the content of linolenic acid and an increase in the content of palmitic acid; the effect was more pronounced in young needles than in fully developed ones (Khan and Malhotra, 1977). Since SO_2 treatment in both types of needles caused reduction in linolenic acid content and an increase in palmitic acid content, it is suggested that SO_2 inhibited both the elongation and desaturation processes. The decline in the linolenic acid content may have occurred by SO_2-stimulated free radical peroxidation reactions, indicated by SO_2-related malonaldehyde accumulation (Khan and Malhotra, 1977). The formation of malonaldehyde also occurs in other plant leaves injured by SO_2 (Peiser and Yang, 1979; Shimazaki et al., 1980).

Carbohydrates and organic acids

Plants exposed to SO_2 exhibit increasing amounts of soluble sugars (Khan and Malhotra, 1977: Malhotra and Sarkar, 1979; Koziol and Jordan, 1978). In Pinus banksiana, SO_2 fumigation

(0.34 and 0.51 ppm) increased the content of the reducing sugars and reduced that of the non-reducing sugars (Malhotra and Sarkar, 1979). It was suggested that the increase was due to a breakdown of polysaccharides rich in reducing sugars. Koziol and Jordon (1978) showed that SO_2 exposure of Phaseolus vulgaris seedlings caused a reduction in starch content. Reduction in non-structural total carbohydrates has also been reported following SO_2 exposure of Ulmus americana seedlings (Constantinidou and Kozlowski, 1979). Recently Bucher-Wallin et al. (1979) found significant increases in the activities of several glycosidases in the foliage of clonal forest trees following exposure to low levels of SO_2, which suggests that the enzymes were involved in the breakdown of polysaccharides. Since polyhydric sugars are known to act as scavengers of free radicals (Asada, 1980), it could well be a mechanism to cope with increasing SO_2 pollution.

Sulphur dioxide also affects a number of enzymes and metabolites involved in organic acid metabolism. Organic acids act as intermediates of a number of metabolic products and maintain cellular pH. Any changes in their metabolism would therefore have an influence on plant growth and yield. In Pinus banksiana needles, SO_2 decreased the content of two major organic acids, namely quinic acid and shikimic acid and increased the content of syringic acid, a minor component (Sarkar and Malhotra, 1979). Reduction of ^{14}C incorporation into organic acids following fumigation of Pinus thunbergii and Pinus densiflora with SO_2 has also been reported (Ishizaki and Hasegawa, 1974). Such changes may be brought about by modification of either the structure or kinetics of the enzymes involved. In Spinacea oleracea, $SO_3^=$ inhibited NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (Ziegler et al., 1976); however, fumigation with SO_2 (0.7 ppm for 1 hour) caused a significant stimulation of the light-activated enzyme. The stimulation was thought to be due to generation of increased SH groups during fumigation (Miszalski and Ziegler, 1979). Low concentrations of SO_2 similarly stimulated the activities of glucose-6-phosphate and isocitrate dehydrogenases, but high SO_2 concentrations appreciably inhibited the two enzymes (Rabe and Kreeb, 1980). A considerable decline has also been reported in NAD-dependent malate dehydrogenase activity of Pinus banksiana seedlings exposed to 0.34 ppm SO_2; isoenzyme analysis showed one less isoenzyme in the SO_2-treated plants compared to the control (Sarkar and Malhotra, 1979). Decreases in NAD and NADP-dependent malate dehydrogenase activity by $SO_3^=$ were shown to be due to $SO_3^=$ effect on the kinetics and multimolecular forms of the enzymes (Ziegler, 1974).

OZONE

Stomatal response

Stomatal control could be responsible for the differential resistances to ozone (O_3) by seedlings (Treshow, 1970). Humidity, which is highly conducive to stomatal opening, caused considerable ozone injury to plants (Otto and Daines, 1969), but little or no injury occurred when stomata were closed (Heath, 1975). In *Phaseolus vulgaris*, however, maximum sensitivity to O_3 was not related to stomatal number or their resistance to the gas at either surface of the leaf (Evans and Ting, 1974a).

Abscisic acid is known to effect stomatal responses and its application reduces O_3 injury to plants (Fletcher *et al.*, 1972; Jeong *et al.*, 1980). Fumigation with O_3 increased ABA content in *Oryza sativa*; the plants resistant to O_3 had higher ABA content than the sensitive ones (Jeong *et al.*, 1980).

Absorption and permeability effects

Moist surfaces in the mesophyll tissues provide the media in which gaseous O_3 can dissolve; however, little is known about O_3 diffusion and its chemical transformation within the tissues. It has been suggested that O_3 diffuses passively due to a concentration gradient similar to that of CO_2 (Heath, 1975). The concentration of O_3 in the plant tissues is affected by its solubility, rate of decomposition, and pH at the site of absorption.

Similar to SO_2, O_3 can also give rise to the superoxide radical (O_2^{-}), which can produce other radicals such as H_2O_2, $^1O_2$, and $OH^{\cdot}$. These radicals can oxidize various cellular metabolites

(Asada, 1980). A number of membrane constitutents such as SH groups, amino acids, proteins, and unsaturated fatty acids are affected by O_3 (Heath, 1975), probably as a result of free radical attack.

Permeability changes in O_3-fumigated tissues are thought to be due to leaky plasmalemma; these include changes in permeability to water (Evans and Ting, 1973), glucose (Dugger and Palmer, 1969; Perchorowicz and Ting, 1974), and ions (Evans and Ting, 1974b; Heath and Frederick, 1979). Permeability of mitochondrial (Lee, 1968) and chloroplast membranes (Nobel and Wang, 1973) is also influenced by O_3. Sutton and Ting (1977) demonstrated that O_3-induced membrane injury can be repaired by energy dependent processes.

Photosynthesis

Fumigation of plants with O_3 inhibited photosynthesis which was reversed about 24 hours after the termination of exposure (Hill and Littlefield, 1969; Pell and Brennan, 1973). The direct effect of O_3 on carboxylases involved in photosynthetic CO_2 fixations are not well know. Thomson et al. (1966) speculated that the granulation of chloroplast stroma seen in electron micrographs of O_3-treated tissues was due to oxidation of SH groups in the Fraction 1 protein (RuDP carboxylase). In Oryza sativa plants, fumigation with O_3 reduced the activity of

RuDP carboxylase in both young and old leaves 12-24 hours after fumigation, but 48 hours after fumigation the enzyme activity had recovered to some extent only in the young leaves (Nakamura and Saka, 1978).

Treatment of isolated Spinacea oleracea chloroplast suspensions with O_3 inhibited electron transport in both photosystems; Photosystem I, however, was more sensitive than Photosystem II (Coulson and Heath, 1974). The treatment inhibited photophosphorylation by reducing electron transport and not by uncoupling. Chang and Heggestad (1974) have also demonstrated the effect of O_3 on Photosystem II in Spinacea oleracea chloroplasts. Inhibition by O_3 of Photosystem II was proportional to the extent of visible injury in relatively resistant (Bel-B) and sensitive (Bel-W_3) Nicotiana tabacum leaves (Rhoads and Brennan, 1978). Ozone treatment of isolated chloroplasts from the two varieties, however, equally inhibited their electron transport activities which suggests that the O_3 effect either was at sites other than the electron transport systems or the resistant species was more capable of repairing the injury.

Schreiber et al. (1978) showed that O_3 (0.3 ppm) altered the fluorescence characteristics in Phaseolus vulgaris long before any visible symptoms of injury appeard. Their data also suggested that a low concentration of O_3 for a long exposure was more injurious than a high concentration for a short exposure. On the basis of specific changes in fluorescence they suggested that the site of O_3 action was within the photosynthetic apparatus. The initial damage was thought to be at the donor site of Photosystem II (H_2O-splitting enzyme system) and not directly on its reaction centre. Increasing O_3 exposures also resulted in the inhibition of electron transport from Photosystem II to Photosystem I.

In addition to influencing the activity of the electron transport system, O_3 affected the chlorophyll content of the treated plants (Leffler and Cherry, 1974). In *Phaseolus vulgaris*, a high correlation was found between chlorophyll loss and visible necrosis (Knudson *et al*., 1977), and the ratio of Chlorophyll *a* to Chlorophyll *b* declined with increasing O_3 injury. Such changes were due either to more O_3 succeptibility of Chlorophyll *a* than Chlorophyll *b* or to their altered biosynthesis. Beckerson and Hofstra (1979c) observed a decrease in Chlorophyll *a* and *b* following exposure of *Phaseolus vulgaris* to O_3.

Respiration

The effect of O_3 on respiration is varibale; it can either stimulate (Todd, 1958; Dugger and Palmer, 1969; Barnes, 1972) or inhibit (MacDowell 1965) plant respiration. Ozone exposure inhibited respiration and phosphorylation in *Nicotiana tabacum* leaf mitochondria (Lee, 1967). In *Phaseolus vulgaris*, there was no effect on respiration immediately after O_3 exposure, but significant stimulation occurred within 24 hours. The content of ATP and total adenylate, however, increased immediately following O_3 exposure (Pell and Brennan, 1973). The effect of O_3 on photorespiration and related metabolic processes is not known at the present time.

Amino Acids and Protein Metabolism

Ozone can cause either an increase or decrease in amino acid content of plants (Ting and Mukerji, 1971; Tomlinson and Rich, 1967; Tingey *et al.*, 1973). Using $^{14}CO_2$, Wilkinson and Barnes (1973) demonstrated an increase in the synthesis of alanaine and serine after exposure of *Pinus strobus* and *Pinus taeda* to O_3. Similarly, the protein content of *Glycine max* leaves increased 24 hours after exposure to O_3 (Tingey *et al.*, 1973), which suggests an increase in the biosynthesis of amino acids and proteins. On the other hand, the protein content of other plants was markedly reduced by O_3 treatment (Ting and

Mukerji, 1971; Constantinidou and Kozlowski, 1979), thereby suggesting either a breakdown of the existing proteins or no synthesis of the new ones.

Chang (1971) reported that in Phaseolus vulgaris leaves, O_3 can cause dissociation of the chloroplast polysomes but not of the cytoplasmic polysomes. It has been suggested that such changes were induced by desiccation brought about by O_3 (Heath, 1975).

Ozone can also affect nitrogen metabolism by inhibiting nitrate reductase in Glycine max leaves; the inhibition of the enzyme, however, occurred only under in vivo and not in vitro conditions (Tingey et al., 1973). This was explained as inhibition in the in vivo formation of reduced NADP (required for the enzyme activity) and not as a direct effect on the enzyme. In Zea mays and Glycine max leaves, O_3 treatment inhibited the activities of both nitrite and nitrate reductases (Leffler and Cherry, 1974). Nitrite reductase, a chloroplast enzyme, was inhibited more severely by O_3 than the cytoplasmic nitrate reductase.

Fatty acid and lipid metabolism

Treshow et al. (1969) reported that exposure to O_3 resulted in a loss of pigmentation and neutral lipids in the fungus Colletotrichum lindemuthianum, but its fatty acid composition remained unaffected. The response in lipids was attributed to an inhibitory effect on their biosynthesis, probably brought about as a result of SH oxidation. On the other hand, recent work with two sensitive cultivars of Nicotiana tabacum showed that fumigations of plants with O_3 (0.25 or 0.30 ppm for 6 hours) significantly increased the lipid content of the leaves but caused a decrease in triglyceride fatty acids (Trevathan et al., 1979). It was speculated that the increase in lipids was an injury response, because rust infection causes a similar accumulation of lipids (Lösel, 1978). Variable effects of O_3 have been reported on fatty acid content in different plant species (Tomlinson and Rich, 1969, 1970, 1971; Swanson et al., 1973). In Nicotiana tabacum leaves, O_3 caused a reduction in all fatty acids, with the largest decreases being in palmitic (16:0) followed by linolenic (18:3) acids (Tomlinson and Rich, 1969). Such a reduction could occur by inhibition of fatty acid synthesising and desaturating enzymes.

Ozone can also affect polyunsaturated fatty acids by oxidative mechanisms. Such oxidations can change the properties of the membranes. Malonaldehyde, an oxidation product of unsaturated fatty acids, is formed upon treatment of unsaturated lipids with O_3 (Mudd et al., 1971b); similarly, increase in malonaldehyde was accompanied by a decrease in unsaturated fatty acid (Frederick and Heath, 1970). Increased production of malonaldehyde has also been observed in leaves of Oryza sativa

(Nakamura and Saka, 1978) and Phaseolus vulgaris (Tomlinson and Rich, 1970) after exposure to O_3.

The direct effect of O_3 on the lipid biosynthesis of isolated chloroplasts has been reported by Mudd et al.(1971a,b). Ozone impaired the ability of chloroplasts to metabolize [1-^{14}C] acetate or acetyl -CoA for lipid biosynthesis. This was attributed to oxidation of essential SH groups (Mudd et al., 1971a). The biosynthesis of galactolipids in chloroplasts was similarly inhibited by O_3 and by other agents that bind SH groups (Mudd et al., 1971b). Ozone treatment, however, did not change the fatty acid composition, which led Mudd et al., (1971a) to suggest that fatty acids of the chloroplast membranes were not very accessible to oxidation by O_3.

Ozone treated plants also exhibit a decrease in free sterols (Tomlinson and Rich, 1971, 1973; Spotts et al., 1975; Menser et al., 1977; Trevathan et al., 1979) and an increase in sterol glycosides and esterified sterol glycosides (Tomlinson and Rich, 1971). These changes may affect membrane permeability.

Carbohydrates and related metobolism

Ozone can either increase (Tingey et al.,.1973. 1976a) or decrease (Miller et al., 1969; Constantinidou and Kozlowski, 1979) the level of soluble sugars and carbohydrates in the leaves of treated plants. Tingey et al. (1973) showed that in Glycine max leaves a single acute exposure of O_3 (0.49 ppm) caused a significant initial decrease in the leaves of reducing sugars, which was followed by a subsequent increase. The starch content did not change, however, which suggests that the initial decrease in soluble sugars could have resulted from a depression in the photosynthetic rate and that the subsequent increase could be due to reduced sugar utilization and/or reduced sugar translocation for carbohydrate synthesis. In Pinus ponderosa, O_3 exposure resulted in an increase in the content of soluble sugars, starch and phenols in the foliage and a decrease in their contents in the roots (Tingey et al., 1976b). Using $^{14}CO_2$, Wilkinson and Barnes (1973) demonstrated that in Pinus strobus and Pinus taeda, O_3 decreased the incorporation of the label into simple soluble sugars while increasing its incorporation into sugar phosphates. Such alterations in the carbohydrate metabolism could be attributed to the effect of O_3 on enzyme activities. A decrease in the activity of glyceraldehyde-3-phosphate dehydrogenase and an increase in glucose-6-phosphate dehydrogenase in Glycine max leaves was attributed to O_3-induced inhibition of the glycolytic pathway and stimulation of the Pentose Phosphate Pathway (Tingey et al., 1975, 1976a). Stimulation of Pentose Phosphate Pathway also occurs in diseased and aged plant tissues (Goodman et al., 1967; Gibbs, 1966).

Since sugars also act as scavengers of free radicals (Asada, 1980), it is possible that an increase in their levels can partly overcome the phytotoxic effects of free radicals produced by O_3. This is a tentative speculation and awaits experimental evidence.

Phenols and related metabolism

Keen and Taylor (1975) found an accumulation of isoflavonoids in O_3-treated plants and suggested that plants under stress can trigger such metabolic responses. In *Medicago sativa*, an accumulation of 4', 7-dihydroxyflavone occurred after exposure to 0.2 ppm O_3 for 2.5 hours, and its concentration markedly increased with increasing symptoms of visible injury (Hurwitz *et al*., 1979). Increased production of isoflavonoids can also occur in response to pathogenic infections (Keen and Kennedy, 1974).

Enzymes involved in phenol metabolism, such as phenylalanine ammonia lyase, polyphenol oxidase and peroxidase, are stimulated by O_3 (Tingey *et al*., 1976a). Activation of these enzymes would stimulate oxidation of phenols to quinones and cause accumulation of their polymerization products. These products could be responsible for necrotic appearance in injured leaves. Peroxidase activity has been reported to increase in plants after exposure to O_3 (Curtis and Howell, 1971; Dass and

Weaver, 1972; Tingey *et al.*, 1975, 1976a; Curtis *et al.*, 1976). Isoenzyme analysis of peroxidase in two cultivars of *Glycine max* with varying sensitivities to O_3 showed that in the resistant cultivar O_3 caused an increase in the activity of a few isoenzymes and in the sensitive cultivar it increased the activity of all isoenzymes (Curtis *et al.*, 1976).

Plants subjected to stress produce elevated levels of ethylene (Abeles, 1973), which was also found in plants exposed to O_3 (Craker, 1971). Ethylene is also a product of normal plant metabolism and has been shown to stimulate the activities of phenylalanine ammonia lyase, polyphenol oxidase, and peroxidase (Abeles, 1973). It is therefore uncertain whether the changes in the activities of these enzymes occur as a direct effect of O_3-induced ethylene production.

OXIDES OF NITROGEN

Stomatal response

Little is known about the effects of oxides of nitrogen (NO_x) on plant stomata and their biochemistry. Because of greater absorption by plants of labelled nitrogen dioxide ($^{15}NO_2$) during the day than at night, it was suggested that the gaseous uptake was dependent on stomatal aperture (Yoneyama *et al.*, 1979; Kaji *et al.*, 1980). High stomatal resistance to NO_2 absorption in the dark was considered to be the reason for decreased NO_2 uptake in Phaseolus vulgaris. NO_2 inhibited transpiration in illuminated leaves, thereby causing partial stomatal closure (Srivastava et al., 1975).

Absorption and biochemical transformations

Formation of nitrate (NO_3^-) and nitrite (NO_2^-) has been demonstrated in plants fumigated with NO_2; initially both NO_3^- and NO_2^- are formed in equal amounts, and this is followed by accumulation of only NO_2^- (Zeevaart, 1976). Nitrite is more toxic than NO_3^- (Mudd, 1973), and in many plants it is detoxified by enzymic mechanisms up to a certain concentration. Plants absorb gaseous NO_2 more rapidly than NO (Bennett and Hill, 1975), mainly because NO_2 reacts rapidly with water, while NO is almost insoluble. Fumigation experiments using labelled NO_2 ($^{15}NO_2$) further demonstrated that absorbed NO_2 gas is easily converted to NO_3^- and NO_2^- before further utilization in plant metabolism (Yoneyama and Sasakawa, 1979; Kaji et al., 1980). The NO_2 injury to plants occurs either as a result of acidification or because of a photooxidation process (Zeevaart, 1976).

Photosynthesis

Decreased photosynthesis has been demonstrated upon exposure of plants to gaseous NO and NO_2, even at concentrations that do not produce visible injury (Hill and Bennett, 1970; Bennett and Hill, 1975; Capron and Mansfield, 1976). The combined effect of the two gases was additive; the NO effect, however was much more rapid than the effect of NO_2 (Hill and Bennett, 1970). Fumigated plants can recover their photosynthetic ability upon transfer to clean air (Hill and Bennett, 1970; Bennett and Hill, 1975). Srivastava et al. (1975) showed that a decrease in photosynthesis in Phaseolus vulgaris was related to NO_2 concentration and length of exposure.

Exposure of Pisum sativum to NO_2 produced little change in RuDP carboxylase activity (Horsman and Wellburn, 1975). Exposure of Pinus banksiana and Picea glauca to low levels of NO_2 (0.2 to 1 ppm for 2 weeks), however, increased RuDP carboxylase activity; the extent of increase was dependent on NO_2 concentration (Khan and Malhotra, 1981c). Short exposures (48 hours) of Pinus banksiana to a relatively high concentration of 2 ppm NO_2 also stimulated the activity of RuDP carboxylase as well as that of glycollate oxidase; the stimulation was, however, greater in glycollate oxidase (174%) than in RuDP carboxylase (112%). Since glycollate oxidase is involved in photorespiration, an increase in its activity greater than that of RuDP carboxylase would imply a drop in photosynthesis. In contrast, the activities of both RuDP carboxylase and glycollate oxidase were markedly inhibited in Betula papyrifera and Alnus crispa, even at a low NO_2 concentration (0.2 ppm) (Khan and Malhotra, 1981c).

Photosynthetic inhibition brought about by NO_x could be explained as competition for NADPH between the processes of nitrite reduction and carbon assimilation in chloroplasts. The acidity produced by NO_2 could also influence electron flow and photophosphorylation. Since photoelectron systems are associated with chloroplast thylakoids, any changes in their structures would influence activities of the photosystems. Nitrogen dioxide has been shown to cause swelling of chloroplast membranes (Wellburn et al., 1972). Biochemical and membrane injury may result if ammonia produced from NO_2 is not rapidly incorporated into amino forms; ammonia has been shown to inhibit photosynthesis by uncoupling electron transport (Avron, 1960) and by inducing structural alterations (Puritch and Barker, 1967).

Little is known concerning NO_x effect on plant pigments involved in photosynthesis. Nash (1976) showed that NO_2 fumigation caused a significant reduction in the chlorophyll content of various lichen species. Unlike lichens, fumigation of Pisum sativum with NO_2 resulted in an increase (10%) in the chlorophyll content (Horsman and Wellburn, 1975). Zeevaart (1976) suggested, on the basis of visible injury, that NO_2 strongly inhibited pigment synthesis in the developing leaves; since development of chlorosis was light-dependent, it is possible that photooxidative processes may have affected the pigments.

Amino acids and proteins

NO_2 has been shown to stimulate amino acid synthesis from $^{14}CO_2$ (Matsushima, 1972). Plants have the ability to metabolize the dissolved NO_x through their NO_3^- assimilation pathway:

$$NO_x \rightarrow NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+ \rightarrow \text{amino acids} \rightarrow \text{Proteins}$$

Plants exposed to gaseous NO_2 can accumulate and metabolize various products of the above pathway (Zeevaart, 1976; Yoneyama and Sasakawa, 1979; Kaji et al., 1980). Induction of nitrate reductase is known to occur by NO_3^- (Beevers and Hageman, 1969); and upon fumigation with NO_2 (Zeevaart, 1974). Stimulation of NO_3^- reductase in fumigated plants causes increased production of NO_2^-, which upon subsequent reduction can be utilized for amino acid synthesis. Reduction of NO_3^- and NO_2^- to NH_4^+ was greatly stimulated in light, which suggests that the process of photosynthesis was the major supplier of the reductant (Zeevaart, 1976).

Isolated chloroplasts with a high rate of CO_2 fixation contain an enzyme system capable of reducing NO_2^- in the presence of light (Magalhaes et al., 1974; Miflin, 1974). The addition of ferredoxin stimulated such a reduction (Beevers and Hageman, 1969). A functional association of Photosystem I and ferredoxin with NO_2^- reduction in the chloroplasts has also been demonstrated (Neyra and Hageman, 1974). Plaut et al. (1977) showed that NO_2^- reduction in Spinacea oleracea chloroplasts was more rapid in the presence of HCO_3^- than in its absence, provided that NO_2^- was added several minutes after HCO_3^-. This led them to suggest that CO_2-fixation products may have regulatory role in NO_2^- reduction.

Using labelled NO_2 ($^{15}NO_2$) it has been shown that most of the absorbed NO_2 was transformed into reduced organic nitrogen compounds; the label was highest in the amide nitrogen of glutamine, followed by glutamate, aspartate, alanine, and amino butyric acid (Yoneyama and Sasakawa, 1979; Kaji et al., 1980). It was thought that following NO_2^- reduction the NH_4^+ was metabolized by glutamine synthetase (GS) and glutamate synthetase (GOGAT) systems. Differences in the distribution of ^{15}N occurred depending on whether plants were fumigated during the day or at night (Kaji et al., 1980). At night the label was present mainly in the amide nitrogen with a small amount in amino acids, while during the day the amino acids synthesis was greatly stimulated. This led to the speculation that light stimulates GOGAT and not GS (Ito et al., 1978; Kaji et al., 1980).

Like NO_2, NO also affected nitrogen metabolizing enzymes. For example, Wellburn et al., (1976) showed that NO fumigation caused an appreciable increase in the activity of nitrite reductase. Later work (Wellburn et al., 1980) showed that NO not only stimulated nitrite reductase but also affected other related enzymes. Fumigation of a tomato (Lycopersicon esculentum) cultivar (Ailsa Craig) sensitive to NO resulted in a significant increase in the activities of glutamate dehydrogenese (GD), glutamate pyruvate transaminase, and glatamate oxaloacetate transaminase (GOT); fumigation of an NO-resistant cultivar (Santo) stimulated only GOT. These results suggest that nitrogen from NO_x could be assimilated by plants through different metabolic systems (GS and GOGAT or GD).

Fatty acids and lipids

In Chlorella pyrenoidosa, NO_2^- (25 mM) markedly inhibited lipid biosynthesis; the inhibition was greater in the dark than in the light (Yung and Mudd, 1966). It is possible that the increased supply of NADPH in the light stimulated reduction of NO_2^- to NH_4^+, which does not affect lipid biosynthesis even in the dark (Yung and Mudd, 1966). Little is known about the effect of NO_x in lipid metabolism in vascular plants. Fumigation of Pinus banksiana seedlings with 2 ppm NO_2 for 48 hours inhibited the biosynthesis of lipids characteristic of chloroplast membranes (phospholipids and galactolipids) long before any visible symptoms of injury appeared (Khan and Malhotra, 1981e).

Oxides of nitrogen have the potential to oxidize unsaturated fatty acids. It is, however, not yet known as to what NO_x concentration in the atmosphere would affect these fatty acids in plant membranes.

Other metabolic processes

Dark respiration in primary leaves of *Phaseolus vulgaris* was more severely inhibited by increasing doses of NO_2 than was photosynthesis, and the inhibition was not reversed quickly upon removal of NO_2 from the atmosphere (Srivastava et al., 1975).

Peroxidase activity was found to be stimulated by NO_2 fumigation. Fumigation of *Pinus banksiana* at 2 ppm for 48 hours stimulated peroxidase activity by 25%, while in *Alnus crispa* the enzyme was stimulated (35%) at a much lower NO_2 dose (0.2 ppm for 24 hours) (Khan and Malhotra, 1981c). No visible symptoms of injury were detected in either *Pinus banksiana* or *Alnus crispa* following these fumigations. Stimulation in peroxidase activity also occurred in plants fumigated with NO (Wellburn *et al*., 1976).

PEROXYACYL NITRATES

Peroxyacetyl nitrate (PAN) is the most commonly studied homologue of peroxyacyl nitrates and is a very phytotoxic component of photooxidative smog.

Similar to other gaseous pollutants, PAN is absorbed mainly through the stomata. In plant leaves its inhibitory effect on photosynthesis is dependent on light (Taylor et al., 1961; Dugger et al., 1963).

Recent work has shown that fumigation conditions can influence the response of photosynthesis in lichens (Sigal and Taylor, 1979). Long exposures at low concentrations of PAN reduced photosynthesis in species such as Parmelia sulcata and Hypogymnia enteromorpha but had no effect on Collema nigrescens. Short fumigations at high concentrations, on the other hand, produced a slight photosynthetic stimulation in all species except Hypogymnia enteromorpha. Dugger et al., (1965) showed that fumigation at a low concentration of PAN for a short duration inhibited O_2 evolution in chloroplasts of treated plants but had no effect on photophosphorylation. Treatment of isolated chloroplasts with PAN, however, inhibited electron transport, photophosphorylation, and CO_2-fixation. Coulson and Heath (1975) also showed that exposure of isolated Spinacea oleracea chloroplasts to PAN inhibited electron transport in both photosystems.

The inhibitory effect of PAN on enzymes has been attributed to its ability to oxidize SH groups in proteins and metabolites such as cysteine, reduced glutathione, CoA, lipoic acid, and methionine (Mudd, 1975b). The oxidizing nature of PAN is not limited to only SH groups, because it can also oxidize NADH and NADPH, which would eventually interfere with metabolic reactions involving these reduced coenzymes. For more-detailed biochemical effects of PAN, refer to reviews by Ziegler (1973b) and Mudd (1975b).

FLUORIDES

Uptake and stomatal response

Fluorides in the air occur in either gaseous or particulate forms. The gaseous form (HF) is absorbed through the leaves, while the particulate form is generally adsorbed on the outer surfaces of the plant and is thus less injurious to plants (Davison and Blakemore, 1976). The development of injury and accumulation of fluoride in plants exposed to air-borne fluorides depend on both biological and environmental factors. Gaseous fluoride absorption through stomata can influence stomatal responses. Poovaiah and Wiebe (1973) demonstrated a partial closure of *Glycine max* stomata after 1 hour of exposure to 0.15 ppm HF; the effect, however, became more severe after 4 hours of exposure. Stomatal opening did not recover compeltely in clean air until the following day. HF fumigation of *Glycine max* also severely depressed the transpiration rate and caused water potential and leaf temperature to increase. Plant uptake of fluoride was greater during the day time fumigations than at night. At present nothing is known about the biochemical basis of such responses.

The site of fluoride accumulation inside the leaf cell is not known with certainty, although it has been suggested that after passing through the cell wall fluoride attacks cytoplasmic membranes and is partially retained there and perhaps transferred to the vacuoles (Treshow, 1971). Chloroplasts have also been suggested as the site of fluoride accumulation in leaves of *Citrus sinensis* (Chang and Thompson, 1966).

Photosynthesis

Exposure of *Gladiolus* to HF caused a decline in photosynthetic CO_2-fixation, but the effect was transient (Thomas and Hendricks, 1956). Little is known about the effect of fluoride on enzymes involved in photosynthetic CO_2-fixation.

At concentrations that did not produce visible injury, HF had no effect on the activity of PEP carboxylase in leaves of *Phaseolus vulgaris* (McCune *et al.*, 1964). Yang and Miller (1963b), on the other hand, reported an increase in the activity of PEP carboxylase and dark CO_2-fixation in fluoride-injured leaves of *Glycine max*.

Potassium fluoride has been shown to inhibit the Hill reaction activity of *Phaseolus vulgaris* chloroplasts (Ballantyne, 1972). Ballantyne (1979) also found, however, that application of fluoride through *Phaseolus vulgaris* petioles had little effect on Hill reaction activity of the leaf chloroplasts. Further work is needed to determine the site of fluoride action in the two photosystems.

The mechanism by which fluorides affect chlorophyll in plant leaves is not clear. Low concentrations of fluoride (1.3 to 12.4 ppb) caused a slight depression of Chlorophyll a and Chlorophyll b; however, the chlorophylls returned to their normal levels after the recovery period (Weinstein, 1961). It has been suggested that fluoride causes a reduction in chlorophyll synthesis (McNulty and Newman, 1961).

Respiration

Fluorides can stimulate or inhibit plant respiration (Treshow, 1971; Chang, 1975). Miller and Miler (1974) observed that, depending on the duration of exposure, HF caused either a stimulation or a depression in intact tissue respiration. Respiratory rates and ATPase activity from isolated mitochondria showed the same pattern as intact tissue respiration, but mitochondrial phosphorylation was severely inhibited regardless of inhibition or stimulation in tissue respiration. Fluoride also stimulated mitochondrial swelling and caused leakage of proteins which suggests that membranes were the primary site of fluoride action. Psenak *et al.* (1977) showed that KF inhibited succinate oxidation more severely than it inhibited malate and NADH oxidations in mitochondria of *Brasica oleracea* and *Glycine max*. The difference was suggested to be due to membrane association of succinoxidase and matrix location of malate oxidase. Respiration changes in fluoride-treated plants could also be attributed to changes in the activities of the oxidative enzymes (Lee *et al*., 1966).

Amino acids and protein metabolism

The amino acid metabolism is markedly affected in plants exposed to fluoride. In leaves of *Glycine max* fumigated with HF, necrosis was accompanied by an increase in free amino acids and asparagine content (Yang and Miller, 1963a). Marked increases in free amino acids and amines (asparagine/glutamine) were also reported in needles of *Picea abies* at all stages of HF-induced injury (Jäger and Grill, 1975). Among the amino acids analyzed HF reduced the content of only alanine, perhaps due to inhibition of enolase activity. It is, however, not certain whether the observed increase in free amino acids was due to increased breakdown of cellular proteins or to inhibition of protein synthesis or to both. Chang (1970 a,b) has shown that NaF caused a decrease in the size of both free and bound ribosomes as well as a decrease in their protein and RNA content. The disorganization of the ribosomal systems was suggested to be due to an increase in the activity of RNAase upon fluoride treatment.

Fatty acids and lipid metabolism

Not much is known about the effect of fluoride on plant fatty acid and lipid metabolism. Yee-Meiler (1975) observed a a significant increase in the activity of non-specific esterase in foliage of Betula verrucosa and Picea abies plants placed at various distances from a fluoride emission source. Increased esterase activity may have an influence on the metabolism of lipids, including those involved in plant membranes. Recently, Simola and Koskimies-Soininen (1980) demonstrated the effect of KF on the fatty acid composition of lipid fractions from Sphagnum fimbriatum gametophytes; the effect was more pronounced in gametophytes grown at 25°C than at 15°C. Fluoride caused an increase in palmitic acid (16:0) and a decrease in linoleic acid (18:2) in all lipid fractions, while linolenic acid (18:3) decreased in glyco-and neutral lipids. The authors suggested that fluoride inhibited elongation of the fatty acids; however, their results showed that KF also caused an increase in stearic (18:0) and oleic (18:1) acids in a number of lipid fractions from material grown at 25°C. It therefore appears that inhibition of chain elongation was not the only event affected by KF and that a decrease in 18:2 and 18:3 fatty acids may also be due to inhibition of the desaturase(s) activity. Such changes in the composition of membrane lipids could influence metabolic functions associated with cellular membranes.

Carbohydrate and organic acid metabolism

Plants fumigated with HF show changes in sugars, polysaccharides, and organic acids contents (Adams and Emerson, 1961; Weinstein 1961; Yang and Miller, 1963a; McCune et al., 1964).

Some changes in the carbohydrates and organic acids can be explained as the direct effects of fluorides on various enzymes involved in the metabolism. Enzymes such as glucose-6-phosphate dehydrogenase (Lee *et al*., 1966), enolase (McCune *et al*., 1964; Lee *et al*., 1966; Miller, 1958), and phosphoglucomutase (Ordin and Altman, 1965) have been shown to be affected by fluoride. Psenak *et al*. (1977) tested the effects of various fluoride compounds on the activity, of three isolated malate dehydrogenase isoenzymes and found that NaF and KF had no effect on the enzyme activity although these compounds inhibited malate oxidation in the mitochondria. Among other fluoride compounds, fluoropyruvic acid also inhibited isolated enzymes. It is possible that, in contrast to isolated enzymes, the sensitivity in the cells and organelles was influenced by other events in the metabolism.

Other metabolic functions

McCune *et al*. (1970) observed that HF had no effect on either the level of acid soluble nucleotides or the distribution of ^{32}P into various nucleotide fractions from leaves of treated plants. Similarly, HF fumigation had no effect on acid soluble phosphorus compounds (Pack and Wilson, 1967). Yee-Meiller (1975) studied the effects of various levels of fluoride on the activity of plant acid phosphatase and found no correlation between the enzyme activity and the extent of plant exposure to fluoride. The acid phosphatase activity in *Pinus banksiana*, however, was severely inhibited by fluoride (Malhotra and Khan, 1980). The effects of fluoride on the activity of acid and alkaline phosphatases have been summarized by Chang (1975).

Keller and Schwager (1971) reported an increase in peroxidase activity of several forest tree species with decreasing distances from a fluoride emission source; the increase in peroxidase activity was related to fluoride content of the tissues. The investigators suggested that this increase was a direct response to high fluoride uptake by the foliage rather than an artifact of fluoride dust interaction with the enzyme during extraction. This reasoning was based on the fact that *in vitro* addition of NaF and CaF_2 had no effect on the enzyme. Increased peroxidase activity is thought to be an indication of premature aging, which could have an effect on growth and yield of vegetation.

POLLUTANT MIXTURES

Stomatal Response

Very little is known about how stomatal regulation and associated biochemical and physiological processes are influenced by pollutant mixtures. The results of some recent studies suggest that stomatal physiology may be affected differently by pollutant mixtures than by a single pollutant. In *Phaseolus vulgaris* the transpiration rates were stimulated by SO_2 and NO_2 individually but were inhibited by the mixture (Ashenden, 1979). Synergistic effects on stomatal closure and visible injury in *Helianthus annuus* have been reported in response to an O_3-NO_2 mixture (Omasa *et al.*, 1980). In a number of agricultural plant species, stomatal resistance increased more in response to an SO_2-O_3 mixture than to those pollutants singly (Beckerson and Hofstra, 1979a, b). In spite of the similarities in stomatal response, these species exhibited considerable differences in the development of symptoms of visible injury, which suggests that the sensitivity was not dependent on only the stomatal behavior. Changes in stomatal responses, would however, influence the absorption of each pollutant from the pollutant mixtures. Elkiey and Ormrod (1980) showed that in *Petunia hybrida*, the amount of SO_2 and O_3 absorbed from a mixture was generally less than that from a single pollutant. Similarly, SO_2 uptake in *Pinus banksiana* and *Betula papyrifera* was less in plants treated with a mixture of SO_2 and NO_2 than in those exposed to SO_2 alone (Khan and Malhotra, 1981c).

Membrane permeability

Membrane permeability has been shown to be affected by pollutants such as SO_2 and O_3 (see earlier sections). Beckerson and Hofstra (1980), using a number of different plant species, demonstrated that the effect of an SO_2-O_3 mixture on membrane permeability is different from that of the same pollutants individually. In *Phaseolus vulgaris* and *Glycine max*, solute leakage increased significantly after O_3 treatment but not after an O_3-SO_2 mixture treatment, which suggests an antagonistic effect of SO_2 on membrane permeability. On the other hand, in Cucumis sativus and Raphanus sativus, membrane permeability increased more after treatment with an O_3-SO_2 mixture than with O_3 alone.

Photosynthesis and other metabolic processes

White et al. (1974) reported that in Medicago sativa photosynthesis was inhibited synergistically at a low concentration of an SO_2-NO_2 mixture (0.15 ppm + 0.25 ppm, respectively) but not at a high concentration (0.5 ppm for both pollutants). Additive effects of an SO_2-NO_2 mixture have been reported on the reduction of photosynthesis in Pisum sativum (Bull and Mansfield, 1974).

Under field conditions where emissions of gaseous pollutants are often accompanied by high levels of CO_2, the effect of a pollutant mixture on photosynthesis could be influenced by the CO_2 concentration in the atmosphere. Hou et al.,(1977) observed that at a high CO_2 concentration, photosynthesis in Medicago sativa was much less inhibited by a mixture of SO_2 and NO_2 than at a low CO_2 concentration. Other environmental conditions can also modify the photosynthetic response to pollutant mixtures; e.g., plants exposed to a mixture of SO_2 and O_3 showed synergistic reduction in photosynthesis, but the effect was greater at low light intensities and high humidity than at high light intensitites and low humidity (Carlson, 1979).

Information about the effects of pollutant mixtures on activities of various enzymes is very limited. Horsman and Wellburn (1975) studied the effect of SO_2 and NO_2 mixtures on various enzymes in Pisum sativum and observed synergistic effects on the activities of RuDP carboxylase and peroxidase. Recent work has also showed that various enzyme activities in several forest tree species exposed to SO_2 and NO_2 mixtures were affected differently from those exposed to single pollutants (Khan and Malhotra, 1981c). These effects were additive (peroxidase and glycollate oxidase in Pinus banksiana and RuDP carboxylase in Alnus crispa), synergistic (peroxidase and glycollate oxidase in Alnus crispa), and antagonistic (peroxidase and glycollate oxidase in Betula papyrifera).

In Ulmus americana, a mixture of SO_2 and O_3 had a different effect on cellular metabolites compared to the effects of these pollutants individually (Constantinidou and Kozlowski, 1979). These effects were either antagonistic, synergistic, or additive, depending upon the type of tissues examined and the length of time after fumigation. Antagonistic effects have also been reported on the destruction of chlorophylls upon treatment with a mixture of SO_2 and O_3 (Beckerson and Hofstra, 1979c). It is therefore obvious that many mixed pollutants can either prevent or accelerate the toxic responses produced by individual pollutants.

REFERENCES

Abeles, F.B. (1973). Ethylene in Plant Biology. Academic Press, New York, pp 302.

Adams, D.F., and Emerson, M.T. (1961). Variations in starch and total polysaccharide content of Pinus ponderosa needles with fluoride fumigation. Plant Physiol., 36, 261-265.

Arndt, U. (1970). Konzentrationsänderungen bei freien Aminosäuren in pflanzen unter dem Einfluss von Fluorwasserstoff and schwefeldioxid. Staub Reinhalt. Luft, 30, 256-259.

Arrigoni, O. (1959). The enzymic oxidation of sulphite in mitochondrial preparations of pea internodes. Ital. J. Biochem. 8, 181-186.

Asada, K. (1980). Formation and scavenging of superoxide in chloroplasts, with relation to injury by sulphur dioxide, in: Studies on the effects of air pollutants on plants and mechanism of phytotoxicity. Res. Rep. Natl. Inst. Environ. Stud., Japan, 11, 165-179.

Asada, K., and Kiso, K. (1973). Initiation of aerobic oxidation of sulphite by illuminated spinach chloroplasts. Eur. J. Biochem., 33, 253-257.

Asada, K., Kiso, K., and Yoshikawa, K. (1974) Univalent reduction of molecular oxygen by spinach chloroplasts on illumination. J. Biol. Chem., 249, 2175-2181.

Asada, K., Kitoh, S., Deura, R., and Kasai, Z. (1965). Effect of α - hydroxysulfonates on photochemical reactions of spinach chloroplasts and participation of glycolate in photophosphorylation. Plant & Cell Physiol., 6, 615-629.

Asada, K., Takahashi, M., Tanaka, K., and Nakano, Y. (1977). Formation of active oxygen and its fate in chloroplasts. in Biochemical and Medical aspects of Active oxygen (Eds. O. Hayaishi and K. Asada), pp 45-63, University of Tokyo Press, Tokyo.

Ashenden, T.W. (1979). Effects of SO_2 and NO_2 pollution on transpiration in Phaseolus vulgaris L. Environ. Pollut., 18, 45-49.

Ashenden, T.W., and Mansfield, T.A. (1977). Influence of wind speed on the sensitivity of ryegrass to SO_2 J. Exp. Bot., 28, 729-735.

Avron, M. (1960). Photophosphorylation by swisschard chloroplasts. Biochem. Biophys. Acta, 40, 257-272.

Ballantyne, D.J. (1972). Fluoride inhibition of the Hill reaction in bean chloroplasts. Atmos. Environ., 6, 267-273.

Ballantyne, D.J. (1973). Sulphite inhibition of ATP formation in plant mitochondria. Phytochemistry, 12, 1207-1209.

Ballantyne, D.J. (1977). Sulfite oxidation by mitochondria from green and etiolated peas. Phytochemistry, 16, 49-50.

Ballantyne, D.J., Johnson, A.M., and Dijak, M. (1979). Physiological effects of systemic fluoride applications to bean (Paseolus vulgaris)leaves and pea (Pisum sativum) epicotyls. Fluoride, 12, 155-162.

Barnes, R.L. (1972). Effects of chronic exposure to ozone on photosynthesis and respiration of pines. Environ. Pollut., 3, 133-138.

Beckerson, D.W., and Hofstra, G. (1979a). Stomatal responses of white bean to O_3 and SO_2 singly or in combination. Atoms. Environ., 13, 533-535.

Beckerson, D.W., and Hofstra, G. (1979b). Response of leaf diffusive resistance of raddish, cucumber and soybean to O_3 and SO_2 singly or in combination. Atoms. Environ., 13, 1263-1268.

Beckerson, D.W. and Hofstra, G. (1979c). Effect of sulphur dioxide, and ozone singly or in combination on leaf chlorophyll, RNA and protein in white bean. Can. J. Bot., 57, 1940-1945.

Beckerson, D.W., and Hofstra, G. (1980). Effects of sulphur dioxide and ozone, singly or in combination, on membrane permeability. Can. J. Bot., 58, 451-457.

Beevers, L., and Hageman, R.H. (1969). Nitrate reduction in higher plants. Annu. Rev. Plant Physiol., 20, 495-522.

Bennett, J.H., and Hill, A.C. (1975). Interaction of air pollutants with canopies of vegetation, in Responses of Plants to Air Pollution (Eds. J.B. Mudd, and T.T. Kozlowski), pp 273-306, Academic Press, New York.

Benson, A.A. (1963). The plant sulfolipid. Adv. Lipid Res., 1, 387-394.

Bisop, N.I. (1958). The influence of the herbicide, DCMU, on the oxygen evolving system of photosynthesis. Biochem. Biophys. Acta , 27, 205-206.

Black, C.R., and Black, V.J. (1979a). The effect of low concentration of sulphur dioxide on stomatal conductance and epidermal cell survival in field bean (Vicia faba L.), J. Exp. Bot., 30, 291-298.

Black, C.R., and Black, V.J. (1979b). Light and scanning electron microscopy of SO_2 induced injury to leaf surfaces of field bean (*Vicia faba* L.). *Plant, Cell and Environment*, 2, 329-333.

Black, V.J., and Unsworth, M.H. (1979a). A system for measuring effects of sulphur dioxide on gas exchange of plants. *J. Exp. Bot.*, 30, 81-88.

Black, V.J., and Unsworth, M.H. (1979b). Effects of low concentrations of sulphur dioxide on gas exchange of plants and dark respiration of *Vicia faba*. *J. Exp. Bot.*, 30, 473-483.

Black, V.J., and Unsworth, M.H. (1980). Stomatal responses to sulphur dioxide and vapour pressure deficit. *J. Exp. Bot.*, 31, 667-677.

Boardman, N.K. (1968). The photochemical systems of photosynthesis. *Adv. Enzymol.*, 30, 1-79.

Bressan, R.A., Wilson, L.G., and Filner, P. (1978). Mechanisms of resistance to sulfur dioxide in cucurbitacea. *Plant Physiol.*, 61, 761-767.

Brunold, C., and Erismann, K.H. (1976). Sulfur dioxide as a sulfur source in duckweeds (*Lemna minor* L). *Experientia*, 32, 296-297.

Bucher-Wallin, I.K., Bernhard, L., and Bucher, J.B. (1979). Einfluss niedriger SO_2-Konzentrationen auf die Aktivität einiger Glykosidasen der Assimilationsorgane verklonter Waldbäume. *Eur. J. For. Path.*, 9, 6-15.

Bull, J.N., and Mansfield, T.A. (1974). Photosynthesis in leaves exposed to SO_2 and NO_2. *Nature*, 250, 443-444.

Capron, T.M., and Mansfield, T.A. (1976). Inhibition of net photosynthesis in tomato in air polluted with NO and NO_2. *J. Exp. Bot.*, 27, 1181-1186.

Caput, C., Belot, Y., Auclair, D., and Decourt, N. (1978). Absorption of sulphur dioxide by pine needles leading to acute injury. *Environ. Pollut.*, 16, 3-15.

Carlson, R.W. (1979). Reduction in the photosynthetic rate of *Acer*, *Quercus* and *Fraxinus* species caused by sulphur dioxide and ozone. *Environ. Pollut.*, 18, 159-170.

Chang, C.W. (1970a). Effect of fluoride on ribosomes from corn roots. Changes with growth retardation. *Physiol. Plant.*, 23, 536-543.

Chang, C.W. (1970b). Effect of fluoride on ribosomes and ribonuclease from corn roots. *Can. J. Biochem.*, 48, 450-454.

Chang, C.W. (1971). Effect of ozone on ribosomes in pinto bean leaves. Phytochemistry, 10, 2863-2868.

Chang, C.W. (1975). Fluorides, in *Responses of Plants to Air Pollution* (Eds. J.B. Mudd and T.T. Kozlowski), pp 57-95, Academic Press, New York.

Chang, C.W., and Thompson, C.R. (1966). Site of fluoride accumulation in novel orange leaves. *Plant Physiol*., 41, 211-213.

Chang, C.W., and Heggestad, H.E. (1974). Effect of ozone on Photosystem II in *Spinacea oleracea* chloroplasts. *Phytochemistry*, 13, 871-873.

Cohen, S.S. (1971). *Introduction to polyamines*, Prentice Hall, Inc., Englewood Cliffs, New Jersey, pp. 179.

Constantinidou, H.A., and Kozlowski, T.T. (1979). Effects of sulfur dioxide and ozone on *Ulmus americana* seedlings. II. Carbohydrates, proteins, and lipids. *Can. J. Bot.*, 57, 176-184.

Cormis, L.D. (1968). Degagement d'hdrogene sulfure par des plantes soumises a une atmosphere contenant de l'anhydride sulfureux. *C.R. Acad. Sci. Ser. D*., 266, 683-685.

Coulson, C., and Heath, R.L. (1974). Inhibition of the photosynthetic capacity of isolated chloroplasts by ozone. *Plant Pysiol.*, 53, 32-38.

Coulson, C.L., and Heath, R.L. (1975). The interaction of peroxyacetyl nitrate (PAN) with the electron flow of isolated chloroplasts. *Atmos. Environ.*, 9, 231-238.

Cowling, D.W., and Bristow, A.W. (1979). Effects of SO_2 on sulfur and nitrogen fractions and on free amino acids in perennial ryegrass. *J. Sci. Food Agric.*, 30, 354-360.

Craker, L.E. (1971). Ethylene production from ozone injured plants. *Environ. Pollut.*, 1, 299-304.

Curtis, C.R., and Howell, R.L. (1971). Increases in peroxidase isoenzyme activity in bean leaves exposed to low doses of ozone. *Phytopathology*, 61, 1306-1307.

Curtis, C.R., Howell, R.L., and Kremer, D.F. (1976). Soybean peroxidases from ozone injury. *Environ. Pollut.*, 11, 189-194.

Das, H.C. and Weaver, G.M. (1972). Enzymatic changes in intact leaves of *Phaseolus vulgaris* following ozone fumigation. *Atmos. Environ.*, 6, 759-763.

Davison, A.W., and Blakemore, J. (1976). Factors determining fluoride accumulation in forage, in *Effects of Air Pollutants* on Plants (Ed. T.A. Mansfied), pp 17-30, Cambridge University Press, Cambridge.

Dittrich, P., Mayer, M., and Meusel, M. (1979). Proton-stimulated opening of stomata in relation to chloride uptake by guard cells. *Planta*, 144, 305-309.

Dugger, W.M., Taylor, O.C., Klein, W.H., and Shropshire, W. (1963). Action spectrum of peroxyacetyl nitrate damage to bean plants. *Nature*, 198, 75-76.

Dugger, W.M., Mudd, J.B., and Koukol, J. (1965). Effect of PAN on certain photosynthetic reactions. *Arch. Environ. Health*, 10, 195-200.

Dugger, W.M., and Palmer, R.L. (1969). Carbohydrate metabolism in leaves of rough lemon as influenced by ozone. *Proc. First Intern. Citrus Symp.*, 2, 711-715.

Elkiey, T., and Ormrod, D.P. (1980). Sorption of ozone and sulfur dioxide by petunia leaves. *J. Environ. Qual.*, 93-95.

Epel, B.L., and Neumann, J. (1973). The mechanism of the oxidation of ascorbate and Mn^{++} by chloroplasts. The role of radical superoxide. *Biochem. Biopys. Acta*, 325, 520-529.

Evans, L.S., and Ting, I.P. (1973). Ozone - induced membrane permeability changes. *Amer. J. Bot.*, 60, 155-162.

Evans, L.S., and Ting, I.P. (1974a). Ozone sensitivity of leaves; Relationship to leaf water content, gas transfer resistance, and anatomical characterstic. *Amer. J. Bot.*, 61, 592-597.

Evans, L.S., and Ting, I.P. (1974b). Effect of Ozone on ^{86}Rb-labeled potassium transport in leaves of *Phaseolus vulgaris* L., *Atmos. Environ.*, 8, 855-861.

Fischer, K. (1971). Methoden zur Erkennung and Beurteilung forstschädlicher Luftverunreinigungen. Chemische und physikalische Reaktionen SO_2-begaster Pflauzen und Blatter. *Mitt. Forstl. Bandes-Versuchsanst. Wien*, 92, 209-231.

Fletcher, R.A., Adedipe, N.O., and Ormrod, D.P. (1972). Abscissic acid protects bean leaves from ozone - induced phytotoxicity. *Can. J. Bot.*, 50, 2389-2391.

Frederick, P.E., and Heath, R.L. (1970). Ozone-induced fatty acid and viability changes in Chlorella. Plant Physiol., 55, 15-19 (1970).

Furukawa, A., Isoda, O., Iwaki, H., and Totsuka, T. (1980a). Interspecific differences in resistance to sulfur dioxide, in Studies on the effect of air pollutants on plants and mechanisms of phytotoxicity . Res. Rep. Natl. Inst. Environ. stud. Japan, 11, 113-126.

Furukawa, A., Natori, T., and Totsuka, T. (1980b). The effect of SO_2 on net photosynthesis in sunflower leaf, in Studies on the effect of air pollutants on plants and mechanisms of phytotoxicity . Res. Rep. Natl. Inst. Environ. Stud., Japan, 11, 1-8.

Garsed, S.G., and Read, D.J. (1977a). Sulphur dioxide metabolism in soybean, Glycine max var Biloxi. 1. The effects of light and dark on the uptake and translocation of $^{35}SO_2$. New Phytol., 78, 111-119.

Garsed, S.G., and Read, D.J. (1977b) Sulphur dioxide metabolism in soybean, Glycine max var Biloxi. II. Biochemical distribution of $^{35}SO_2$ products. New Phytol., 99, 538-592.

Garsed, S.G., and Read, D.J. (1977c). The uptake and metabolism of $^{35}SO_2$ in plants of differing sensitivity to sulphur dioxide. Environ. Pollut., 13, 173-186.

Gerwick, B.C., Ku, S.B., and Black, C.C. (1980). Initiation of sulfate activation. A variation in C_4 photosynthesis plants. Science, 209, 513-515.

Gezeilus, K., and Hallgren, J.E. (1980). Effect of SO_3^{2-} on the activity of ribulose biphosphate carboxylase from seedlings of Pinus silvestris. Physiol. Plant., 49, 354-358.

Gibbs, M. (1966). Carbohydrates: Their role in plant metabolism and nutrition, in Plant Physiology (Ed. F.C. Steward) IVB, PP 3-115. Academic Press, New York.

Gilbert, O.L. (1968). Biological indicators of air pollution. Ph.D thesis, Univ. Newcastle upon Tyne.

Godzik, S., and Li skens, H.F. (1974). Concentration changes of free amino acids in primary leaves after continuous and interrupted SO_2 fumigation and recovery. Environ. Pollut., 7, 25-38.

Goodman, R.N., Kiraly, Z., and Zaitlin, M. (1967). The Biochemistry and Physiology of infectious Plant Disease, D. VanNostrand Co. Princeton, pp 354.

Grill, D., Esterbauer, H., and Welt, R. (1979a). Einfluss von SO_2 auf das Ascorbinsäuresystem der Fichtennadeln. Phytopath. Z., 96, 361-368.

Grill, D., Esterbauer, H., and Klosch (1979b). Effect of sulphur dioxide on glutathione in leaves of plants. Environ. Pollut., 19, 187-194.

Grill, D., Esterbauer, H., Scharner, M., and Felgitsch, Ch. (1980). Effect of sulfur dioxide on protein-SH in needles of Picea abies. Eur. J. For. Pathol., 10, 263-267.

Guderian, R. (1977). Air pollution. Phytotoxicity of acid gases and its significance in air pollution control, in Ecological studies (Eds. W.D. Billings, F. Golley, O.L. Lange, and J.S. Olson) 22, 127 pp Springer Verlag, Berlin.

Hallgren, J.E. (1978). Physiological and biochemical effects of sulphur dioxide on plants,, in Sulphur in the Environment: Part II Ecological Impact (Ed. J.O. Nriagu), pp 163-209, John Wiley & Sons, New York.

Hampp, R., and Ziegler, I. (1977). Sulfate and sulfite translocation via the phosphate translocator of the inner envelop membrane of chloroplasts. Planta, 137, 309-312.

Heath, R.L. (1975). 'Ozone , in Responses of plants to air pollution (Eds. J.B. Mudd and T.T. Kozlowski) pp 23-55, Academic Press, New York.

Heath, R.L., and Frederick, P.E. (1979). Ozone alteration of membrane permeability in Chlorella. Plant Physiol., 64, 455-459.

Hill, A.C., and Littlefield, N. (1969). Ozone effect on apparent photosynthesis, rate of transpiration and stomatal closure in plants. Environ. Sci. Technol., 3, 52-56.

Hill, A.C. and Bennett, J.H. (1970). Inhibition of apparent photosynthesis by nitrogen oxides. Atmos. Environ., 4, 341-348.

Horsman, D.C., and Wellburn, A.R. (1975). Synergistic effect of SO_2 and NO_2 polluted air upon enzyme activity in pea seedlings. Environ. Pollut., 8, 123-133.

Hou, L.Y., Hill, A.C., and Soleimani, A. (1977). Influence of CO_2 on the effects of SO_2 and NO_2 on alfalfa. Environ. Pollut., 12, 7-16.

Hurwitz, B., Pell, E.J., and Sherwood, R.T. (1979). Status of coumestrol and 4',7-dihydroxyflavone in alfalfa foliage exposed to ozone. Phytopathology, 69, 810-813.

Ishizaki, A., and Hasegawa, M. (1974). Physiological and morphological properties of the Japanese black and red pine trees (Pinus thunbergii parl, and P. densiflora Sieb. et Zucc) on the resistance to air pollution. Bull Fac. Agric. Tamagawa Univ. 14, 40-50 (in Japanese with English summary).

Ito, O., Yoneyama, T., and Kumazawa, K. (1978). Amino acid metabolism in plant leaf IV. The effect of light on ammonium assimilation and glutamine metabolism in the cells isolated from spinach leaves. Plant & Cell Physiol., 19, 1109-119.

Jäger, H.J., and Grill, D. (1975). Einfluss von SO_2 Und HF auf freie Aminosauren der Fichte (Picea abies [L.] Karsten). Eur. J. For. Path., 5, 279-286.

James, A.T., and Nichols, B.W. (1966). Lipids of photosynthetic systems. Nature, 210, 372-375.

Jensen, K.F., and Kozlowski, T.T. (1975). Absorption and translocation of sulfur dioxide by seedlings of four forest tree species . J. Environ. Qual., 4, 379-382.

Jeong, Y.H., Nakamura, H., and Ota, Y. (1980). Physiological studies on photochemical oxidants injury in rice plants. 1. Varietal difference of abscisic acid content and its relation to the resistance to ozone. Jap. J. Crop Sci. 49, 456-460 (in Japanese with English summary).

Jiracek, V., Machackova, I., and Kastir, J. (1972). Nachweis der Bisulfite - Addukte (α -Oxysulfonäuren) von Carbonylverbindungen in den mit SO_2 behandelten Erbsenkeimlingen. Experientia, 28, 1007-1009.

Kaji, M., Yoneyama, T., Tosuka, T., and Iwaki, H. (1980). Absorption of atmospheric NO_2 by plants and soils VI. Transformation of NO_2 absorbed in the leaves and transfer of the nitrogen through the plants, in Studies on the effects of air pollutants on plants and mechanisms of phytotoxicity . Res. Rep. Natl. Inst. Environ. Stud., Japan, 11, 51-58.

Keen, N.T., and Kennedy, B.W. (1974). Hydroxyphaseollin and related isoflavonoids in the hypersensitive resistant response of soybeans against Pseudomonas glycinea. Physiol. Plant Pathol., 4, 173-185.

Keen, N.T., and Taylor, O.C. (1975). Ozone injury in soybeans. Isoflavonoid accumulation is related to necrosis.. Plant Physiol., 55, 731-733.

Keller, Th., (1976). Auswirkungen niedriger SO_2-Konzentrationen auf junge Fichten. Schweiz. Z. Forstwes., 127, 237-251.

Keller, Th., and Schwager, H. (1971). Der Nachweis unsichtbarer (,,Phsiologischer") Fluor - Immissionsschädigungen an Waldbäumen durch eine einfache kolorimetrische Bestimmung der Peroxidase - Aktivität. Eur. J. For. Path., 1, 6-18.

Keller, Th., Schwager, H., and Yee Meiler, D. (1976). Der Nachweis Winterlicher SO_2-Immissionen an jungen Fichten. Ein vergleich dreier Methoden. Eur. J. For. Pathol., 6, 244-249.

Keller, Th., and Schwager, H. (1977). Air pollution and ascorbic acid. Eur. J. For. Pathol., 7, 338-350.

Khan, A.A., and Malhotra, S.S. (1977). Effects of aqueous sulphur dioxide on pine needle glycolipids. Phytochemistry, 16, 539-543.

Khan, A.A., and Malhotra, S.S. (1978) Biosynthesis of lipids in chloroplasts isolated from jack pine needles. Phytochemistry, 17, 1107-1110.

Khan, A.A. and Malhotra, S.S. (1981a). Studies on ribulose diphosphate carboxylase and glycollate oxidase from needles of Pinus banksiana: Effects of sulphur dioxide and metals on enzyme activities. Manuscript in preparation.

Khan, A.A., and Malhotra, S.S. (1981b). Sulphite oxidation by chloroplast and cytoplasmic fractions of Pinus banksiana needles. Manuscript in preparation.

Khan, A.A., and Malhotra, S.S. (1981c). Effects of SO_2, NO_2 and their mixture on the enzymic activities of forest plant species. Manuscript in preparation.

Khan, A.A., and Malhotra, S.S. (1981d). Protein biosynthesis in pine needles and its inhibition by sulphur dioxide. Manuscript in preparation.

Khan, A.A., and Malhotra, S.S. (1981e). Response of NO_2 fumigation on lipid biosynthesis in pine needles. Manuscript in preparation.

Knudson, L.L., Tibbitts, T.W., and Edwards, G.E. (1977). Measurement of ozone injury by determination of leaf chlorophyll concentration. Plant Physiol., 60, 606-608.

Kondo, N., and Sugahara, K. (1978) hanges in transpiration rate of SO_2-resistant and - sensitive plants with SO_2 fumigation and the participation of abscisic acid. Plant & Cell Physiol., 19, 365-373.

Kondo, N., Akiyama, Y., Fujiwara, M., and Sugahara, K. (1980a). Sulfite oxidizing activities in plants, in Studies on the effects of air pollutants on plants and mechanisms of phytotoxicity. Res. Rep. Natl. Inst. Environ. Stud. Japan, 11, 135-150.

Kondo, N., Maruta, I., and Sugahara, K. (1980b). Abscisic acid-dependent changes in transpiration rate with SO_2 fumigation and the effect of sulfite and pH on stomatal aperture, in Studies on the effects of air pollutants on plants and mechanisms of phytotoxicity. Res. Rep. Natl. Inst. Environ. Stud. Japan, 11, 127-136.

Koziol, M.J., and Jordan, C.F. (1978). Changes in carbohydrate levels in red kindney bean (*Phaseolus vulgaris* L.) exposed to sulphur dioxide. *J. Exp. Bot.*, 29, 1037-1043.

Koziol, M.J., and Cowling, D.W. (1978). Growth of ryegrass (*Lolium perenne* L) exposed to SO_2. *J. Exp. Bot.*, 29, 1431-1439

Lauenroth, W.K., Black, C.J., and Dodd, J.L. (1979). Sulfur accumulation in western wheatgrass exposed to three controlled SO_2 concentrations. *Plant and Soil*, 53, 131-136.

Lee, C., Miller, G.W., and Welkie, G.W. (1966). The effects of hydrogen fluoride and wounding on respiratory enzymes in soybean leaves. *Air Water Pollut. Int. J.*, 10, 169-181.

Lee, T.T. (1967). Inhibition of oxidative phosphorylation and respiration by ozone in tobacco mitochoudria. *Plant Physiol.*, 42, 691-696.

Lee, T.T. (1968). Effect of ozone on swelling of tobacco mitochondria. *Plant Physiol.*, 43, 133-139.

Leffler, H.R., and Cherry, J.H. (1974). Destruction of enzymatic activities of corn and soybean leaves exposed to ozone. *Can. J. Bot.*, 43, 677-685.

Lehmann, J., and Benson, A.A. (1964). The plant sulfolipid. IX. Sulfosugar synthesis from methyl hexoseenides. *J. Amer. Chem. Soc.*, 86, 4469-4472.

Libera, W., Ziegher, H., and Ziegler, I. (1973). Forderung der Hill Reaktion und der CO_2-Fixierung in isolierten Spinatchloroplasten durch niedere Sulfitkonzentratinen. *Planta*, 109, 269-279.

Lösel, D.M. (1978). Lipid metabolism of leaves of *Poa pratensis* during infection by *Puccinia poarum*. *New Phytol.*, 80, 167-174

MacDowell, F.D.H. (1965). Stages of ozone damage to respiration of tobacco leaves. *Can. J. Bot.*, 43, 419-427.

Magalhaes, A.C., Neyra, C.A., and Hageman, R.H. (1974). Nitrite assimilation and amino nitrogen synthesis in isolated spinach chloroplasts. *Plant Physiol.*, 53, 411-415.

Majernik, O., and Mansfield, T.A. (1970). Direct effect of SO_2 pollution on the degree of opening of stomata. *Nature*, 227, 377-378.

Majernik, O., and Mansfield, T.A. (1971). Effects of SO_2 pollution on stomatal movements in *Vicia faba*. *Phytopathol. Z.*, 71, 123-218.

Majernik, O., and Mansfield, T.A. (1972). Stomatal responses to raised atmospheric CO_2 concentrations during exposure of plants to SO_2 Pollution. *Environ. Pollut.*, 3, 1-7.

Malhotra, S.S. (1976). Effects of sulphur dioxide on biochemical activity and ultrastructural organization of pine needle chloroplasts. New Phytol., 76, 239-245.

Malhotra, S.S. (1977). Effects of aqueous sulphur dioxide on chlorophyll destruction in Pinus contorta. New Phytol., 78, 101-109.

Malhotra, S.S., and Hocking, D. (1976). Biochemical and cytological effects of sulphur dioxide on plant metabolism New Phytol., 76, 227-237.

Malhotra, S.S., and Khan, A.A. (1978). Effects of sulphur dioxide fumigation on lipid biosynthesis in pine needles. Phytochemistry, 17, 241-244.

Malhotra, S.S., and Sarkar, S.K. (1979). Effects of sulphur dioxide on sugar and free amino acid content of pine seedlings. Physiol. Plant., 47, 223-228.

Malhotra, S.S., and Khan, A.A. (1980). Effects of sulphur dioxide and other air pollutants on acid phosphatase activity in pine seedlings. Biochem. Physiol. Pflanzen, 175, 228-236.

Malhotra, S.S., and Khan, A.A. (1981a). Peroxidase activity as an indicator of SO_2 injury to Pinus banksiana and Betula papyrifera. Manuscript in preparation.

Malhotra, S.S., and Khan, A.A. (1981b). Sensitivity of various metabolic processes to sulphur dioxide in an epiphytic lichen, Evernia mesomorpha. Manuscript in preparation.

Mansfield, T.A., and Majernik, O. (1970). Can stomata play a part in protecting plants against air pollutants? Environ. Pollut., 1, 149-154.

Matsushima, J. (1972). Influence of SO_2 and NO_2 on assimilation of amino acids, organic acids and sacchariod in Citrus natsudaidai seedlings. Bull. Fac. Agr., Mie Univ., 44, 131-139.

McCord, J.M. and Fridovich, I. (1969). Superoxide dismutase: an enzymic function of erythrocuprein. J. Biol. Chem., 244, 6049-6055.

McCune, D.C., Weinstein, L.H., Jacobson, J.S., and Hitchcock, A.E. (1964). Some effects of atmospheric fluoride on plant metabolism. J. Air Pollut. Contr. Assoc., 14, 465-468.

McCune, D.C., Weinstein, L.H., and Mancini, J.F. (1970). Effects of hydrogen fluoride on the acid soluble nucleotide metabolism of plants. Contrib. Boyce Thompson Inst., 24, 213-226.

McNulty, I.B., and Newman, D.W. (1961). Mechanisms of fluoride induced chlorosis. Plant Physiol., 36, 385-388.

Menser, H.A., and Heggestad, H.E. (1966). Ozone and sulfur dioxide synergism: injury to tobacco plants. Science 153, 424-425.

Menser, H.A., Chaplin, J.F., Cheng, A.L.S., and Sorokin, T. (1977). Polyphenols, polysterols, and reducing sugars in air-cured tobacco leaves injured by ozone air pollution. Tob.Sci., 21, 35-38.

Miflin, B.J. (1974). Nitrite reduction in leaves; studies in isolated chloroplasts. Planta, 116, 187-196.

Miller, G.W. (1958). Properties of enolase in extracts from pea seed. Plant physiol., 33, 199-206.

Miller, J.E., and Miller, G.W. (1974). Effects of fluoride on mitochondrial activity in higher plants. Physiol. Plant., 32, 115-121.

Miller, J.E., and Xerikos, P. (1979). Residence time of sulfite in SO_2 "sensitive" and "tolerant" soybean cultivars. Environ. Pollut., 18, 259-264.

Miller, P.R., Parmeter, J.R., Flick, B.H., and Martinez, C.W. (1969). Ozone dose response of ponderosa pine seedlings. J. Air Pollut. Cont. Assoc., 19, 435-438.

Miszalski, Z., and Ziegler, I. (1979). Increase in chloroplastic thiol groups by SO_2 and its effect on light modulation of NADP-dependent glyceraldehyde 3-phosphate dehydrogenase. Planta, 145, 383-387.

Miszalski, Z., and Ziegler, H. (1980). "Available SO_2"- a parameter for SO_2 toxicity. Phytopath.Z., 97, 144-147.

Mudd, J.B. (1973). Biochemical effects of some air pollutants on plants. Adv. Chem. Ser. 122, 31-47.

Mudd, J.B. (1975a). Sulfur dioxide, in Responses of Plants to Air Pollution (Eds. J.B. Mudd and T.T. Kozlowski), pp 9-22, Academic Press, New York.

Mudd, J.B. (1975b). Peroxyacyl nitrates, in Responses of Plants to Air Pollution (Eds. J.B. Mudd and T.T. Kozlowski), pp. 97-119, Academic Press, New York.

Mudd, J.B., McManus, T.T., and Ongun, A. (1971a). Inhibition of lipid metabolism in chloroplasts by ozone. Proc. Int. clean Air Congr., 2nd, 1970, pp 256-260, Academic Press, New York.

Mudd, J.B., McManus, T.T., Ongun, A., and McCollogh, T.E. (1971b). Inhibition of glycolipid biosynthesis in chloroplasts by ozone and sulfhydryl reagent. Plant Physiol., 48, 335-339.

Mukerji, S.K., and Yang, S.F. (1974). Phosphoenolpyruvate carboxylase from spinach leaf tissue. Inhibition by sulfite ion. Plant Physiol., 53, 829-834.

Nakamura, H., and Saka, H. (1978). Photochemical oxidants injury in rice plants. III. Effect of ozone on physiological activities in rice plants. Jap. J. Crop Sci., 47, 707-714 (in Japanese with English summary).

Nash, T.H. (1976). Sensitivity of lichens to nitrogen dioxide fumigations. Bryologist, 79, 103-106.

Neyra, C.A., and Hageman, R.H. (1974). Dependence of nitrite reduction on electron transport in chloroplasts. Plant Physiol., 54, 480-483.

Nobel, P.S., and Wang, C.T. (1973). Ozone increases the permeability of isolated pea chloroplasts. Arch. Biochem. Biophys., 157, 388-394.

Oku, T., Shimazaki, K., and Sugahara, K. (1980). Resistance of spruce seedlings to sulfur dioxide fumigation, in Studies on the effects of air pollutants on plants and mechanisms of phytotoxicity. Res. Rep. Natl. Inst. Environ. Stud., Japan, 11, 151-154.

Omasa, K., Abo, F., Natori, T., and Totsuka, T. (1980). Analysis of air pollutant sorption by plants, in Studies on the effects of air pollutants on plants and mechanisms of phytotoxicity . Res. Rep. Natl. Inst. Environ. Stud. Japan, 11, 213-224.

Ordin, L., and Altman, A (1965). Inhibition of phosphoglucomutase activity in oat coleoptiles by air pollutants. Physiol. Plant., 18, 790-797.

Osmond, C.B., and Avadhani, P.N. (1970). Inhibition of the β-carboxylation pathway of CO_2 fixation by bisulfite compounds. Plant Physiol., 45, 228-230.

Otto, H.W., and Daines, R.H. (1969). Plant injury by air pollutants. Influence of humidity on stomatal apertures and plant response to ozone. Science, 163, 1209-1210.

Outlaw, W.H. Jr., and Kennedy, J. (1979). Enzymic and substrate basis for the anaplerotic step in guard cells. Plant Physiol., 62, 648-652.

Pack, M.R., and Wilson, A.M. (1967). Influence of hydrogen fluoride fumigation on acid soluble phosphorus compounds in bean seedlings. Environ. Sci. Technol., 1, 1011-1013.

Pahlich, E. (1971). Allosterische Regulation der Aktivität der Glutamate-hydrogenase aus Erbsenkeimlingen durch das Substrat α-Ketoglutarsäure. Planta, 100, 222-227.

Pahlich, E. (1973). Uber den Hemm - Mechanismus mitochondrialer Glutamat - Oxalacetat - Transaminase in SO_2 begasten Erbsen. Planta, 110, 267-278.

Pahlich, E., Jäger, H.J., and Steubing, L. (1972). Beeinflussung der Aktivitäten von Glutamatdehydrogenase und Glutaminsynthetase aus Erbsenkeimlingen durch SO$_2$. *Angew. Botanik*. 46, 183-197.

Paul, J.S., and Bassham, J.A. (1978). Effects of sulfite on metabolism in isolated mesophyll cells from *Papaver somniferum*. *Plant Physiol.*,62, 210-214.

Peiser, G.D., and Yang, S.F. (1977). Chlorophyll destruction by the bisulfite-oxygen system. *Plant Physiol.* 60, 277-281.

Peiser, G.D., and Yang, S.F. (1978). Chlorophyll destruction in the presence of bisulfite and linoleic acid hydroperoxide. *Phytochemistry*, 17, 79-84.

Peiser, G.D., and Yang, S.F. (1979). Ethylene and ethane production from sulfur dioxide injured plants. *Plant Physiol.*, 63, 142-145.

Pell, E.J., and Brennan, E. (1973). Changes in respiration, photosynthesis, *adenosine 5'-triphosphate*, and total adenylate content of ozonated pinto bean foliage as they relate to symptom expression. *Plant Physiol.*, 51, 378-381.

Perchorowicz, J.T., and Ting, I.P. (1974). Ozone effects on plant cell permeability. *Amer. J. Bot.*, 61, 787-793.

Petering, D.H., and Shih, N.T. (1975). Biochemistry of bisulfite-sulfur dioxide. *Environ. Res.*, 9, 55-65.

Plaut, Z., Lendzian, K., and Bassham, J.A. (1977). Nitrite reduction in reconstituted and whole spinach chloroplasts during carbon dioxide reduction. *Plant Physiol.*, 59, 184-188.

Poovaiah, B.W., and Wiebe, H.H. (1973). Influence of hydrogen fluoride fumigation on the water economy of soybean plants. *Plant Physiol.*, 51, 396-399.

Priebe, A., Klein, H., and Jäger, H.J. (1978). Role of polyamines in SO$_2$-polluted pea plants. *J. Exp. Bot.*,29, 1045-1050.

Psenak,M., Miller, G.W., Yu, M.H., and Lovelace, C.J. (1977). Separation of malic dehydrogenase isoenzymes from soybean tissue in relation to fluoride treatment. *Fluoride*, 10, 63-72.

Puckett, K.J., Nieboer, E., Flora, W.P., and Richardson, D.H.S. (1973). Sulphur dioxide: Its effects on photosynthetic ^{14}C fixation in lichens and suggested mechanisms of phytotoxicity. *New Phytol.*, 72, 141-154.

Puritch, G.S., and Barker, A.V. (1967). Structure and function of tomato leaf chloroplasts during ammonium toxicity. *Plant Physiol.*, 42, 1229-1238.

Pahlich, E., Jäger, H.J., and Steubing, L. (1972). Beeinflussung der Aktivitäten von Glutamatdehydrogenase und Glutaminsynthetase aus Erbsenkeimlingen durch SO_2. Angew. Botanik. 46, 183-197.

Paul, J.S., and Bassham, J.A. (1978). Effects of sulfite on metabolism in isolated mesophyll cells from Papaver somniferum. Plant Physiol.,62, 210-214.

Peiser, G.D., and Yang, S.F. (1977). Chlorophyll destruction by the bisulfite-oxygen system. Plant Physiol., 60, 277-281.

Peiser, G.D., and Yang, S.F. (1978). Chlorophyll destruction in the presence of bisulfite and linoleic acid hydroperoxide. Phytochemistry, 17, 79-84.

Peiser, G.D., and Yang, S.F. (1979). Ethylene and ethane production from sulfur dioxide injured plants. Plant Physiol., 63, 142-145.

Pell, E.J., and Brennan, E. (1973). Changes in respiration, photosynthesis, adenosine 5'-triphosphate, and total adenylate content of ozonated pinto bean foliage as they relate to symptom expression. Plant Physiol., 51, 378-381.

Perchorowicz, J.T., and Ting, I.P. (1974). Ozone effects on plant cell permeability. Amer. J. Bot., 61, 787-793.

Petering, D.H., and Shih, N.T. (1975). Biochemistry of bisulfite-sulfur dioxide. Environ. Res., 9, 55-65.

Plaut, Z., Lendzian, K., and Bassham, J.A. (1977). Nitrite reduction in reconstituted and whole spinach chloroplasts during carbon dioxide reduction. Plant Physiol., 59, 184-188.

Poovaiah, B.W., and Wiebe, H.H. (1973). Influence of hydrogen fluoride fumigation on the water economy of soybean plants. Plant Physiol., 51, 396-399.

Priebe, A., Klein, H., and Jäger, H.J. (1978). Role of polyamines in SO_2-polluted pea plants. J. Exp. Bot.,29, 1045-1050.

Psenak,M., Miller, G.W., Yu, M.H., and Lovelace, C.J. (1977). Separation of malic dehydrogenase isoenzymes from soybean tissue in relation to fluoride treatment. Fluoride, 10, 63-72.

Puckett, K.J., Nieboer, E., Flora, W.P., and Richardson, D.H.S. (1973). Sulphur dioxide: Its effects on photosynthetic ^{14}C fixation in lichens and suggested mechanisms of phytotoxicity. New Phytol., 72, 141-154.

Puritch, G.S., and Barker, A.V. (1967). Structure and function of tomato leaf chloroplasts during ammonium toxicity. Plant Physiol., 42, 1229-1238.

Rabe, R., and Kreeb, K.H. (1980). Wirkungen von SO_2 auf die Enzymaktivität in Pflauzenblättern. *Z. Pflanzenphysiol.*, 97, 215-226.

Radmer, R.J., and Kok, B. (1976). Photoreduction of O_2 primes and replaces CO_2 assimilation. *Plant Physiol.* 58, 336-340.

Rao, D.N., and LeBlanc, B.F. (1965). Effects of sulfur dioxide on the lichen alga, with special reference to chlorophyll. *Bryologist*, 69, 69-75.

Raschke, K. (1975). Stomatal action. *Annu. Rev. Plant Physiol.* 26, 309-340.

Raschke, K. (1979). Movements of stomata, in *Plant Movements, Encyclopedia of Plant Physiology* (Eds. W. Haupt and M.E. Feinleib), pp 383-441, Springer-Verlag, Berlin.

Rhoads, A., and Brennan, E. (1978). The effect of ozone on chloroplast lamellae and isolated mesophyll cells of sensitive and resistant tobacco selections. *Phytopathology*, 68, 883-886.

Ryrie, I.J., and Jagendorf, A.T. (1971). Inhibition of photophosphorylation in chloroplasts by inorganic sulfate. *J. Biol. Chem.*, 246, 582-588.

Sarkar, S.K., and Malhotra, S.S. (1979). Effects of SO_2 on organic acid content and malate dehydrogenase activity in jack pine needles. *Biochem. Physiol. Pflanzen.*, 174, 438-445.

Schiff, J.A., and Hodson, R.C. (1973). The metabolism of sulfate. *Annu. Rev. Plant Physiol.* 24, 381-414.

Schreiber, U., Vidaver, W., Runeckles, V.C., and Rosen, P. (1978). Chlorophyll fluorescence assay for ozone injury in intact plants. *Plant Physiol.*, 61, 80-84.

Schwenn, J.D., Depka, B., Hennies, H.H. (1976). Assimilatory sulfate reduction in chloroplasts: Evidence for the participation of both stromal and membrane-bound enzymes. *Plant & Cell Physiol.*, 17, 165-171.

Sekiya, J., Wilson, L.G., and Filner, P. (1980). Positive correlation between H_2S emission and SO_2 resistance in cucumber. *Plant Physiol. Suppl.*, 65, Abs. 407.

Shaw, A.B., Anderson, M.M., and McCarty, R.E. (1976). Role of galactolipids in spinach chloroplast lamellar membranes. II. Effects of galactolipid depletion on phosphorylation and electron flow. *Plant Physiol.*, 57, 724-729.

Shimazaki, K., and Sugahara, K. (1980a). Specific inhibition of photosystem II activity in chloroplasts by fumigation of spinach leaves with SO_2, in *Studies on the effects of air pollutants on plants and mechanisms of phytotoxicity*. *Res. Rep. Natl. Inst. Environ. Stud.* Japan, 11, 69-77.

Shimazaki, K., and Sugahara, K. (1980b). Inhibition site in electron transport system in chloroplasts by fumigation of lettuce leaves with SO_2, in Studies on the effects of air pollutants on plants and mechanisms of phytotoxicity . Res. Rep. Natl. Inst. Environ. Stud. Japan, 11, 79-89.

Shimazaki, K., Sakaki, T., and Sugahara, K. (1980). Active oxygen participation in chlorophyll destruction and lipid peroxidation in SO_2-fumigated leaves of spinach, in Studies on the effects of air pollutants on plants and mechanisms of phytotoxicity . Res. Rep. Natl. Inst. Environ. Stud., Japan, 11, 91-101.

Sigal, L.L., and Taylor, O.C. (1979). Preliminary studies of the gross photosynthetic response of lichens to peroxyacetyl nitrate fumigations. Bryologist, 82, 564-575.

Silvius, J.E., Ingle, M., and Baer, C.H. (1975). Sulfur dioxide inhibition of photosynthesis in isolated spinach chloroplasts. Plant Physiol., 56, 434-437.

Silvius, J.E., Baer, C.H., Dodrill, S., and Patrick, H. (1976). Photoreduction of sulfur dioxide by spinach leaves and isolated spinach chloroplasts. Plant Physiol., 57, 799-801.

Simola, L.K., and Koskimies-Soininen, K. (1980). The effect of fluoride on the growth and fatty acid composition of Sphagnum fimbriatum at two temperatures. Physiol. Plant., 50, 74-77.

Smith, F.A., and Raven, J.A. (1979). Intracellular pH and its regulation. Annu. Rev. Plant Physiol., 30, 289-311.

Smith, T.A., and Sinclair, C. (1967). The effect of acid feeding on amine formation in barley. Ann. Bot., 31, 103-111.

Soldatini, G.F., Ziegler, I., and Ziegler, H. (1978). Sulfite: Preferential sulfur source and modifier of CO_2 fixation in chlorella vulgaris. Planta, 225-231.

Soldatini, G.F., and Ziegler, I. (1979). Induction of glycolate oxidase by SO_2 in Nicotiana tabacum., Phytochemistry, 18, 21-22.

Spotts, R.A., Lukezic, F.L., and LaCasse, N.L. (1975). The effect of benzimidazole, cholesterol, and a steroid inhibitor on leaf sterols and ozone resistance of bean. Phytopathology, 65, 45-49.

Squire, G.R., and Mansfield, T.A. (1972). A simple method of isolating stomata on detached epidermis by low pH treatment: observation of the importance of subsidiary cells. New Phytol., 71, 1033-1043.

Srivastava, H.S., Jolliffe, P.A., and Runeckles, V.C. (1975). Inhibition of gas excahnge in bean leaves by NO_2. Can. J. Bot., 53, 466-474.

Stokes, D.M., and Walker, D.A. (1971). Phosphoglycerate as a Hill oxidant in reconstituted chloroplast system. Plant Physiol., 48, 163-165.

Sugahara, K., Uchida, S., and Takimoto, M., (1980). Effects of sulfite ions on water soluble chlorophyll proteins, in Studies on effects of air pollutants on plants and mechanisms of phytotoxicity. Res. Rep. Natl. Inst. Environ. Stud., Japan, 11, 103-112.

Sutton, R., and Ting, I. (1977). Evidence for the repair of ozone-induced membrane injury. Amer. J. Bot., 64, 404-411.

Swanson, E.S., Thomson, W.W., and Mudd, J.B. (1973). The effect of ozone on leaf cell membranes. Can. J. Bot., 51, 1213-1219

Tager, J.M., and Rautanen, N. (1956). Sulfite oxidation by plant mitochendrial system. Enzymic and non-enzymic oxidation. Physiol. Plant., 9, 665-673.

Tanaka, H., Takanashi, T., and Yatazawa, M. (1972a). Experimental studies on sulfur dioxide injuries in higher plants. I. Formation of glyoxylate bisulfite in plant leaves exposed to sulfur dioxide. Water, Air, and Soil Pollution, 1, 205-211

Tanaka, H., Takanashi, T., Kadota, M., and Yatazawa, M. (1972b). Experimental studies on sulfur dioxide injuries in higher plants II. Disturbance of amino acid metabolism in plants exposed to sulfur dioxide. Water, Air, and Soil Pollution, 1, 343-346.

Tanaka, K., and Sugahara, K. (1980). Role of superoxide dismutase in the defense against SO_2 toxicity and induction of superoxide dismutase with SO_2 fumigation, in Studies on the effects of air pollutants on plants and mechanisms of phytotoxicity. Res. Rep. Natl. Inst. Environ. Stud., Japan. 11, 155-164.

Taylor, O.C., Dugger, W.M., Cardiff, E.A., and Darley, E.F. (1961). Interaction of light and atmospheric photochemical products (SMOG) within plants. Nature, 192, 814-816.

Thomas, M.D. (1951). Gas damage to plants. Annu. Rev. Plant Physiol. 2, 293-322.

Thomas, M.D., and Hill, G.R. (1935). Absorption of sulfur dioxide by alfalfa and its relation to leaf injury. Plant Physiol., 10, 291-307.

Thomas, M.D., Hendricks, R.H., Collier, T.R., and Hill, G.R. (1943). The utilization of sulfate and sulfur dioxide for the nutrition of alfalfa. Plant Physiol., 18, 343-371.

Thomas, M.D., and Hendricks, R.H. (1956). Effect of air pollution on plants, in Air Pollution Handbook (Eds. P.L. Magill, F.R. Holden, and C. Ackley) p 44, McGraw-Hill, New York.

Thomson, W.W., Dugger, W.M. Jr., and Palmer, R.L. (1966). Effect of ozone on the fine structure of the palisade parenchyma cells of bean leaves. Can. J. Bot., 44, 1677-1682.

Ting, I.P., and Mukerji, S.K. (1971). Leaf ontogeny as a factor in susceptibility to ozone: Amino acid and carbohydrate changes during expansion. Amer. J. Bot., 58, 497-504.

Tingey, D.T., Fites, R.C., and Wickliff, C. (1973). Ozone alteration of nitrate reduction in soybean. Physiol. Plant., 29, 33-38.

Tingey, D.T., Fites, R.C., and Wickliff, C. (1975). Activity changes in selected enzymes from soybean leaves following ozone exposure. Physiol. Plant., 33, 316-320.

Tingey, D.T., Fites, R.C., and Wickliff, C. (1976a). Differential foliar sensitivity of soybean cultivars to ozone associated with differential enzyme activities. Physiol. Plant, 37, 69-72.

Tingey, D.T., Wilhour, R.G., and Standley, C. (1976b). The effect of chronic ozone exposures on the metabolite content of ponderosa pine seedlings. Forest Sci., 22, 234-241.

Todd, G.W. (1958). Effect of ozone and ozonated 1-hexene on respiration and photosynthesis of leaves. Plant Physiol., 33, 416-420.

Tomlinson, H., and Rich, S. (1967). Metabolic changes in free amino acids of bean leaves exposed to ozone. Phytopathology, 57, 972-974.

Tomlinson, H., and Rich, S. (1969). Relating lipid content and fatty acid synthesis to ozone injury of tobacco leaves. Phytopathology, 59, 1284-1286.

Tomlinson, H., and Rich, S. (1970). Lipid peroxidation, a result of injury in bean leaves exposed to ozone. Phytopathology, 61, 1531-1532.

Tomlinson, H., and Rich, S. (1971). Effect of ozone on sterols and sterol derivatives in bean leaves. Phytopathology, 61, 1404-1405.

Tomlinson, H., and Rich, S. (1973). Anti-senescent compounds reduce injury and steriod changes in ozonated leaves and their chloroplasts. Phytopathology, 63, 903-906.

Treshow, M. (1970). Ozone damage to plants. Environ. Pollut., 1, 155-161.

Treshow, M. (1971). Fluorides as air pollutants affecting plants. Annu. Rev. Phytopathol., 9, 21-44.

Treshow, M., Harner, F.M., Price, H.E., and Kormelink, J.R. (1969). Effects of ozone on growth, lipid metabolism, and sporulation of fungi. Phytopathology, 59, 1223-1225.

Trevathan, L.E., Moore, L.D., and Orcutt, D.M. (1979). Symptom expression and free sterol and fatty acid composition of flue-cured tobacco plants exposed to ozone. Phytopathology, 69, 582-585.

Trown, P.W. (1965). An improved method for the isolation of carboxydismutase. Probable identity with Fraction-1 protein and the protein moiety of protochlorophyll holochrome. Biochemistry, 4, 908-918.

Weinstein, L.H. (1961). Effects of atmospheric fluoride on metabolic constituents of tomato and bean leaves. Contrib. Boyce Thompson Inst., 21, 215-231.

Wellburn, A.R., Majernik, O., and Wellburn, F.A.M. (1972). Effects of SO_2 and NO_2 polluted air upon the ultrastructure of chloroplasts. Environ. Pollut., 3, 37-49.

Wellburn, A.R., Capron, T.M., Chan, H.S., and Horsman, D.C. (1976). Biochemical effects of atmospheric pollutants on plants, in Effects of Air Pollutants on Plants (Ed. T.A. Mansfield), pp 106-114, Cambridge Univ. Press, London.

Wellburn, A.R., Wilson, J., and Aldrige, P.H. (1980). Biochemical responses of plants to nitric oxide polluted atmospheres. Environ. Pollut., 22, 219-228.

White, K.L., Hill, A.C., and Bennett, J.H. (1974). Synergistic inhibition of apparent photosynthesis rate of alfalfa by combinations of sulfur dioxide and nitrogen dioxide. Environ. Sci. Tech., 8, 575-576.

Wilkinson, T.G., and Barnes, R.L. (1973). Effects of ozone on $^{14}CO_2$ fixation patterns in pine. Can. J. Bot., 51, 1573-1578.

Wyss, H.R., and Brunold, C. (1979). Regulation of adenosine 5'-phosphosulfate sulfotransferase activity by H_2S and cyst(e)ine in primary leaves of Phaseolus vulgaris L. Planta, 147, 37-42.

Wyss, H.R., and Brunold, C. (1980). Regulation of adenosine 5'-phosphosulfate sulfotransferase by sulfur dioxide in primary leaves of beans (Phaseolus vulgaris). Physiol. Plant., 50, 161-165.

Yang, S.F., and Miller, G.W. (1963a). Biochemical studies on the effect of fluoride on higher plants. 1. Metabolism of carbohydrates, organic acids and amino acids. Biochem. J., 88, 509-516.

Yang, S.F., and Miller, G.W. (1963b). Biochemical studies on the effect of fluoride on higher plants. 3. The effect of fluorine on dark carbon dioxide fixation. Biochem. J., 88, 517-522.

Yang, S.F., and Saleh, M.A. (1973). Destruction of indole-3-acetic acid during the aerobic oxidation of sulfite. Phytochemistry, 12, 1463-1466.

Yee-Meiler, D. (1975). Über die Eignung von Phosphotase-und Esteraseaktivitätsbestimmungen an Fichtennadeln und Birkenblättern Zum Nachweis,,Unsichtbarer Physiologischer" Fluorimissionsschädigungen. Eur. J. For Path., 5, 329-338.

Yoneyama, T., and Sasakawa, H. (1979). Transformation of atmospheric NO_2 absorbed in spinach leaves. Plant & Cell Physiol., 20, 263-266.

Yoneyama, T., Sasakawa, H., and Ishizuka, S. (1979). Absorption of atmospheric NO_2 by plants and soils. II. Nitrite accumulation, nitrite reductase activity and diurnal change of NO_2 absorption in leaves. Soil Sci. Plant Nutr., 25, 267-275.

Yung, K.H., and Mudd, J.B. (1966). Lipid synthesis in the presence of nitrogenous compounds in chlorella pyrenoidosa. Plant Physiol., 41, 506-509.

Zeevaart, A.J. (1974). Induction of nitrate reductase by NO_2. Acta Bot. Neerl., 23, 345-346.

Zeevaart, A.J. (1976). Some effects of fumigating plants for short periods with NO_2. Environ. Pollut., 11, 97-108.

Zelitch, I. (1957). α-Hydroxysulfonates as inhibitors of the enzymatic oxidation of glycolic and lactic acids. J. Biol. Chem., 224, 251-260.

Zelitch, I. (1971). Photosynthesis, Photorespiration and Plant Productivity. Academic Press, New York, pp. 347.

Ziegler, I. (1972). The effect of $SO_3^=$ on the activity of ribulose-1,5-diphosphate carboxylase in isolated spinach chloroplasts. Planta, 103, 155-163.

Ziegler, I. (1973a). Effect of sulphite on phosphoenolpyruvate carboxylase and malate formation in extracts of Zea mays. Phytochemistry, 12, 1027-1030.

Ziegler, I. (1973b). The effect of air polluting gases on plant metabolism, in *Environmental Quality and safety, Vol. II. Global Aspects of Chemistry, Toxicology and Technology as* applied to the Environment (Eds. F. Coulson and F. Korte), pp. 182-208, Academic Press, New York.

Ziegler, I. (1974). Action of sulphite on plant malate dehydrogenase. *Phytochemistry*, 13, 2411-2416.

Ziegler, I. (1975). The effect of SO_2 pollution on plant metabolism. *Residue Rev.*, 56, 79-105.

Ziegler, I. (1977a). Subcellular distribution of ^{35}S-sulfur in spinach leaves after application of $^{35}SO_3^{2-}$, and $^{35}SO_2$. Planta, 135, 25-32.

Ziegler, I. (1977b). Sulfite action on ribulosediphosphosphate carboxylase in the lichen *Pseudevernia furfuracea*. *Oecologia*, 29, 63-66.

Ziegler, I. Marewa, A., and Schoepe, E. (1976). Action of sulphite on the substrate kinetics of chloroplastic NADP-dependent glyceraldehyde 3-phosphate dehydrogenase. *Phytochemistry*, 15, 1627-1632.

Ziegler, I., and Hampp, R. (1977). Control of $^{35}SO_4^{2-}$ and $^{35}SO_3^{2-}$ incorporation into spinach chloroplasts during photosynthetic CO_2 fixation. *Planta*, 137, 303-307.

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GENERAL AND ECOLOGICAL PUBLICATIONS

BIOMONITORING IN THE ATHABASCA OIL SANDS AREA OF ALBERTA: PROGRESS AND PITFALLS

by

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BIOMONITORING IN THE ATHABASCA OIL SANDS AREA OF ALBERTA: PROGRESS AND PITFALLS

1. INTRODUCTION

Biomonitoring is a technique whereby biological samples are used both as indicators of pollution impingement and as a measure of air pollution impact. Obviously, the state of health of any organisms is the best measure of air pollution impact on that species. Biomonitoring also may utilize specific processes or formations of the ecosystem. As with an organism, a process such as decomposition of a plant community response may be the most effective way to assess pollutant impact to these ecosystem components.

Several plant groups, particularly mosses and lichens, have been shown to be sensitive to as well as very efficient in taking up and storing pollutants (Clough 1974; Richardson and Nieboer 1981). If the ecosystem in an area can provide a reliable and consistent measure of both impingement and impact of a specific pollutant or pollutant mixture, and if this is the objective, the expense of establishing and maintaining high technology monitoring instrumentation can be eliminated. This does not mean that air quality measurements will be out of date, quite the opposite. These types of measurements along with deposition studies will still be essential for pollution regulation and testing the reliability of biomonitoring techniques.

Several studies have attempted to use components of the terrestrial ecosystem as indicators of pollution deposition and impact in the Athabasca Oil Sands area. These studies have included the examination of particular groups of organisms such as insects (Hilchie and Ryan 1980) or lichens (Douglas and Skorepa 1976; Addison and Pockett

1980) as well as soils (Takyi and Nyborg 1977; Baker 1980) and vegetation (Addison 1980a; 1980b). The Athabasca Oil Sands area is northeastern Alberta is of particular interest in biomonitoring technique development owing to both the potential for long-range transport of air pollutants to the Province of Saskatchewan and the large amount of information on air quality and pollution deposition that has been generated by the Alberta Oil Sands Environmental Research Program.

This report describes biomonitoring techniques using soils and vegetation in the Athabasca Oil Sands area from 1975 to 1981. It is hoped that the information describing responses to air pollution in the vicinity of Suncor Inc., will serve as an indication of soil and plant monitors that have, and have not proved successful. This is important, particularly at this time when the Research Management Division of Alberta Environment is embarking on a biomonitoring research program. It is expected that with the history of biomonitoring in the area, the program concentrating on technique development may produce an effective biomonitoring system that can be used throughout much of this province and elsewhere.

1.1 STUDY AREA

All work was carried out in the study area as defined by the Alberta Oil Sands Environmental Research Program (Figure 1). The sites selected for detailed examination and manipulation were as close as possible to Suncor Inc., with a reference site at Fort MacKay, about 25 km north of the source (Figure 2). Sites included the five gradient sites (A to E), Fina Airstrip (F), Steepbank (G), Suncor Clearcut (H), Syncrude Turnoff (I), Mildred Lake Research Facility (J) and Fort MacKay (K). A substantial amount of time was spent in an attempt to select sites that were as similar as possible. Stand densities, types, and physical characteristics of soils as well as the composition of the vegetative components of these sites showed minor variation. It was imperative, however, to assume that the sites

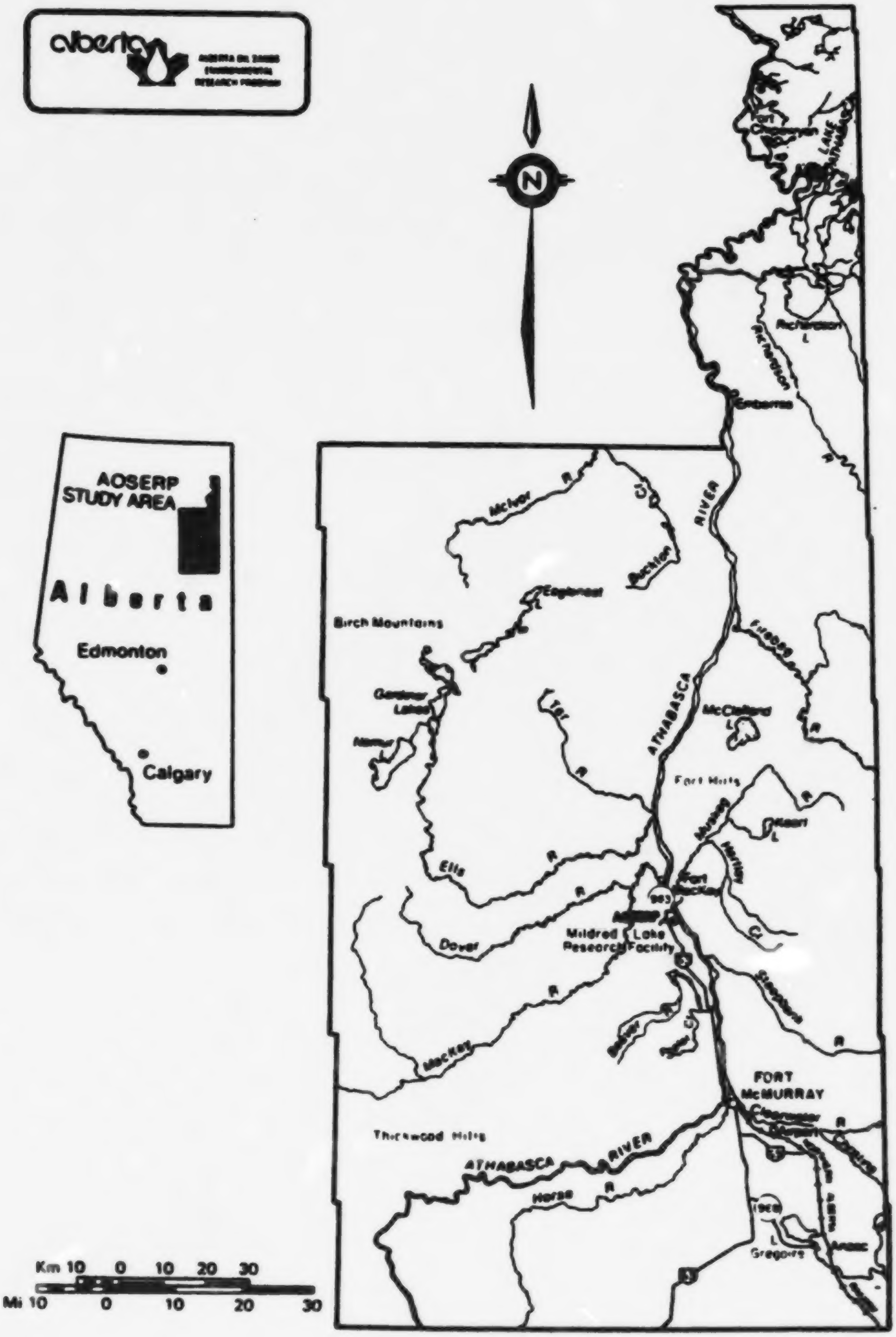

Figure 1. Location of the Alberta Oil Sands Environmental Research Program study area.

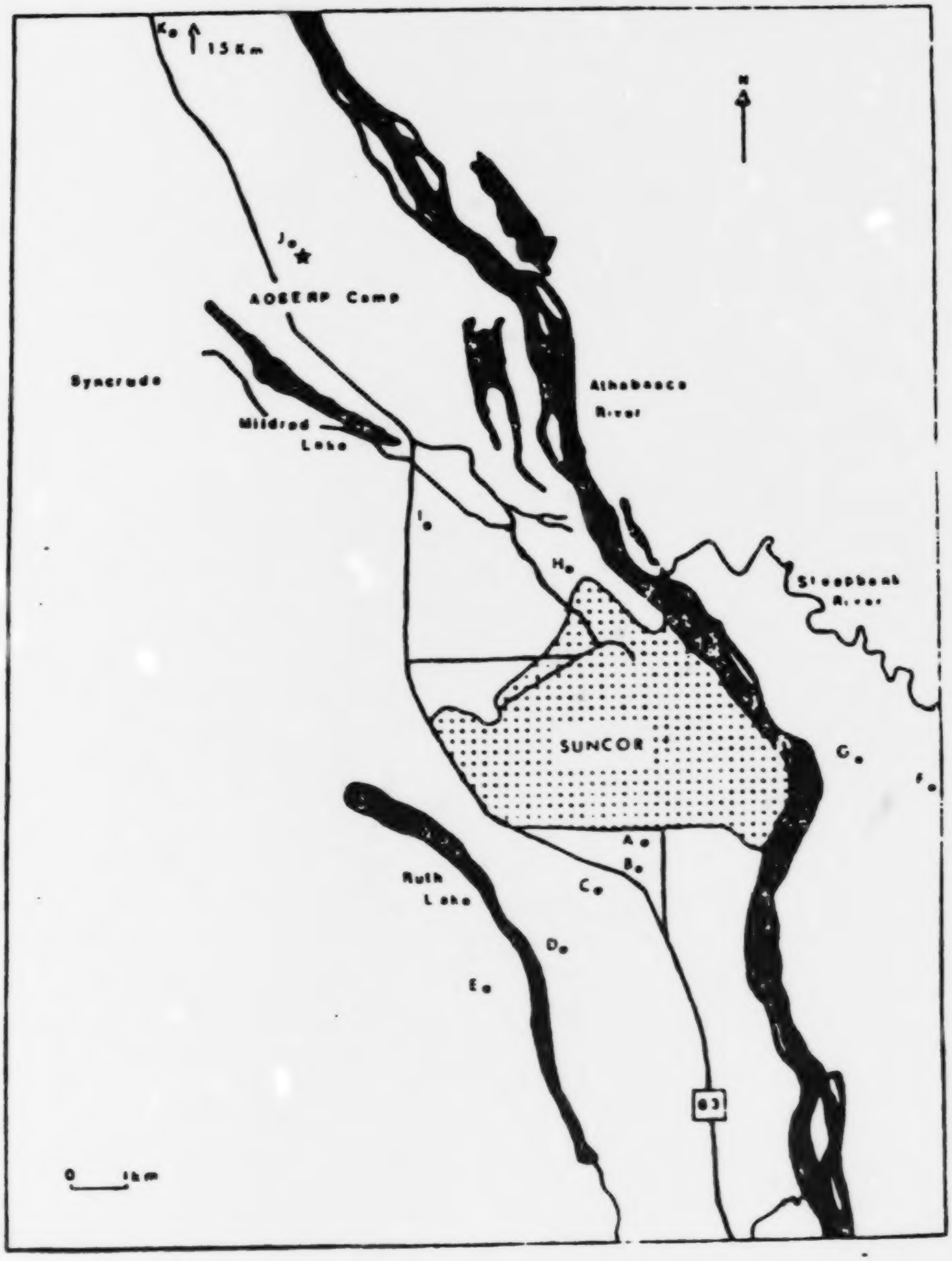

Figure 2. Location of gradient sites and other specific sites used to test biomonitoring techniques.

were different and to quantify the differences that did exist. This was done to avoid the pitfall of making the unjustified and the theoretically unsound assumption that any two forest stands were identical.

2. METHODS AND MATERIALS

2.1 MEASUREMENT OF DEPOSITION

2.1.1 Physical Collectors

2.1.1.1 Precipitation. Three methods were used to monitor deposition of pollutants by both dry-fall and precipitation at sites A to E. In 1977, six cylindrical collectors (20 cm in diameter x 50 cm long) were placed in natural clearings at each site. Mineral oil was placed in each collector to prevent evaporation of the collected precipitation and the trapped material was collected monthly. In 1978, triplicate collectors were placed at the top of the canopy. In this case, a funnel was used to channel precipitation and particulates to a fibre-glass filter and then through a column of two ion exchange resins (one for cations and one for anions). The liquid was then trapped in a reservoir and the entire collector was retrieved after four months. In 1979, the same collector and locations used in 1978 were established with the exception that both the filter and the two exchange resins were eliminated. Samples were collected after four months and concentrated before analysis.

2.1.1.2 Sulphation. Huey plates, 10 cm in diameter, were placed, in duplicate, in three microenvironments (in the open, under jack pine and under white spruce) at sites A to E in the summer of 1977 and at sites A to G (excluding B), J and K from May 1978 to September 1979. Plates were mounted upside down to shield the reactive lead dioxide surface from precipitation. Plates were replaced and analyzed approximately monthly in the summer and every second month in winter.

Sulphation plates at a site close to Suncor Inc. (A) were placed in three different forest stands (jack pine, aspen, and black spruce). Duplicate plates were situated 3 m above the canopy, at the top of the canopy and at various heights (10, 5, 1.5, and 0.7 m). Plates were replaced on a 1 to 2 month interval.

2.1.2 Native Plants

Six species or species groups were sampled at sites A to E and analyzed for S, Al, Fe, P, and K. The species included jack pine (*Pinus banksiana* Lamb.), white spruce (*Picea glauca* (Moench) Voss), common labrador tea (*Ledum groenlandicum* Oeder), common bearberry (*Arctostaphylos uva-ursi* (L.) Spreng.), ground lichens (mainly *Pleurozium schreberi* (Brid.) Mitt.). These six species were selected because they demonstrated the greatest uptake of S close to the pollution source of the 15 species analyzed in 1976 (Addison, unpublished). These species also represented both dry and moist microenvironments. Five replicates were collected for each species. Pine and spruce samples consisted of 1-yr old material collected 7 to 10 m above ground, whereas other samples were of indeterminant age. Duplicate plant samples were passed through a 20 mesh screen and mineralized using an oxygen flask combustion technique (Chan 1975). Element analysis was carried out as described below.

2.1.3 Soils

Duplicate soil samples were collected from each horizon of the profile at each site. The soils were oven dried (105°C for 24 h) and digested using an aluminum block digester (Technicon Inc.) and teflon beakers. Samples (0.5 g) were predigested in 10 mL of 16N HNO_3 and 2 mL of 10N $HClO_4$ for 1.5 h at 70°C. Silica was then driven off with 20 mL of 29N HF at 150°C. The sample was taken to near dryness, dissolved into 1 mL of 12N HCl and brought to 50 mL with glass distilled water. Digests were analyzed for Al, Fe, and K with an atomic absorption spectrophotometer (Instrumentation Laboratories #251), P with an autoanalyzer, and S using a modified Johnson-Nishita method (Carson et al. 1972).

2.2 MEASUREMENT OF RESPONSE

2.2.1 Growth and Reproduction of Jack Pine

2.2.1.1 Basal Area. Radial increment growth of jack pine was determined at five sites (A, G, H, J, and K; Figure 2) by taking two cores from the south and west sides of trees (5 to 10) at each site. The increment for each of the previous 20 years was measured with an Addo-X Tree Ring Measuring Instrument, and the data from the two cores were averaged. Age (taken at 30 cm above the ground) of the trees sampled was also determined. Only cores that went through the centre of the tree were retained.

2.2.1.2 Shoot Length and Leaf Number. At a site close to the pollution source (Site A) and at Site J, about 10 km distant, five branches from the top one-third of each of five trees were collected. The length (distance between bud scale scars in mm) and the number of needles were determined for each of five age classes on each branch.

2.2.1.3 Seed Germination. The effect of air pollutants on jack pine seed viability was determined by collecting and testing seeds from mature (i.e., 35 year old) trees at sites A to E. Seed germination tests were carried out in the laboratory by placing 3 to 5 replicates of 50 seeds on moist filter paper at 20°C (day) and 16°C (night) fluctuating temperatures in the light. Germination was recorded after three weeks.

2.2.1.4 Biochemistry and Physiology. On several occasions during the summer of 1977, triplicate samples of both jack pine and white spruce branches were collected at sites A, C, and E and brought to the laboratory for biochemical and physiological investigation. The tissue was examined for phosphatase and malate dehydrogenase activities, chlorophyll/phaeophytin ratio, total sulphur content, net photosynthesis, and dark respiration. Methods followed were those of Malhotra and Khan (1979).

2.2.2 Plant Community

At each gradient site (A to E) in 1977, a 20 x 20 m plot was established and stand density and age as well as the cover and frequency of the lower stratum vascular plant species were determined. The lower stratum was quantified using 20 1 x 1 m quadrats that were randomly selected from a 20 x 20 matrix of the plot. The sample area represented 5% of the total and quadrat size was greater than the minimum sampling area required as defined by Cain and deCastro (1959). All quadrats were mapped so that future measurements of the site would be as close as possible to the first.

In 1979, these sites were re-examined and the cover of each species in the same quadrats was again estimated. Sites were subsequently compared with themselves and the change over time recorded.

2.2.3 Studies of Naturally Occurring Lichen

2.2.3.1 Condition and Element Content. Samples of *Evernia mesomorpha* and *Hypogymnia physodes* were collected from 69 locations in the vicinity of Suncor Inc. operations (Addison and Puckett 1980). These two species were selected because they were the most common corticolous lichens in the area and represented foliose and fruticose growth forms. Samples of both species were collected from the bark of *Picea mariana* (Mill.) BSP. For each site, only material in the best physical collection was collected. A qualitative estimate of thallus condition on a scale of 1 (least) to 5 (most luxuriant) was made on the samples of *E. mesomorpha* collected for analysis. No such estimate was attempted for *H. physodes* since changes in this lichen morphology and colouration were too subtle to quantify. Tissue samples were oven dried at 80°C to constant weight (24 h). No attempt was made to remove surface deposits on the lichens, but all remnants of the substrate were removed.

All lichen material was frozen in liquid nitrogen and ground to pass through a 60-mesh sieve. Approximately 0.1 g of material was mineralized using oxygen flask combustion and S was determined as above. Potassium was determined on the same samples by atomic absorption. Aluminum, titanium, and vanadium contents were determined on ground, non-ashed samples by instrumental neutron activation analysis.

2.2.3.2 Biochemical Condition. Samples of *E. mesomorpha* were collected in September from sites A, C, E, J, and K (Figure 2). Samples were almost dry at the time of collection in the field. They were brought to the laboratory and kept at -15°C in the dark in polyethylene bags until analysis of acid phosphatase, protein biosynthesis, and ^{14}C bicarbonate incorporation.

2.2.4 Studies of Lichen Transplants

2.2.4.1 Community. A branch lichen transplant study was initiated in 1976. Branches of black spruce were collected at Fort MacKay and relocated in five microsites at each of four locations (Sites H to K, Figure 2). Two branches were placed in a clearing and under jack pine, white and black spruce, and aspen at a height of 1.5 m. Quantification of the cover and frequency of the species groups present were accomplished using a 15 x 20 cm gray card and photographs. Each branch was marked and labelled and the lichens moistened slightly so that they were pliable. Spruce branches are oriented mainly on a plane and, hence, there was no difficulty in placing the quadrat below the branch. Lichens normally grow on the top and sides of branches and, hence, the entire stand was included in a photograph taken from directly above the branch.

Analysis of the photographs was done by projecting the transparency onto a 45 x 60 cm rear projection screen. The screen was divided into 100 units and the presence of each lichen species or species group was recorded for each unit. A correlation was made based on the average cover that each species had when it occurred in a unit.

Lichen community transplants were established at sites A to E in 1977 under spruce trees and the transplants (10 at each site) were quantified in the same manner as described. All transplants were re-examined each year between 1976 and 1980.

2.2.4.2 Element Content and Physiology. Pine branches bearing mainly *Evernia mesomorpha* were placed under jack pine trees. Six replicates were used at each site and material was collected from these transplants, brought into the laboratory and analyzed for total sulphur and available and total K^+ and Mg^{++}. Available K^+ and Mg^{++} was extracted by 1N HCl during shaking for 1 h. Analyses were done by atomic absorption spectrophotometry.

3. RESULTS AND DISCUSSION

3.1 DEPOSITION

3.1.1 Physical Collectors

3.1.1.1 Precipitation. Not one of the attempts to measure deposition through the collection of precipitation was successful. In 1977, damage to the collectors by bears was so extensive that, on several occasions, it was not possible to find a single collector intact after one month. The few samples that were collected were not analyzed since it would serve little purpose with the amount of missing data.

In 1978, collectors on the top of trees resolved the animal damage problem. In spite of much testing in the laboratory and a demonstrated capability of the exchange resins to trap and hold the elements of interest, it was not possible to rely on the results from this collector. After four months of exposure in the field, the resins showed some breakdown and released some of their trapped elements as well as some resin components.

In 1979, no pattern of pollutant deposition could be detected using samples of precipitation collected at the top of the forest canopy throughout the snow-free period (Addison 1980b). Several sites, however, had high levels of certain elements such as copper and sodium relative to the rest of the sites indicating contamination of the sample during processing. Further work is required on this technique before reliable results can be assured.

3.1.1.2 Sulphation Plates. Sulphation plates demonstrated that both distance and the type of canopy were important in determining the amount of SO_2 present at any location. At all sites, plates located in the open absorbed the greatest amount of SO_2, followed by those located under pine. In most cases, the amount of SO_2 absorbed by the plate under spruce was half to two-thirds that absorbed in the open (Table 1). The effect of canopy was significant ($p < 0.05$) in all months.

Table 1. Sulphur deposition ($mg \cdot m^{-2} . d^{-1}$) in the vicinity of the Suncor Inc. operations in the Athabasca Oil Sands area in 1979.

Site	Distance (km) and Direction from Suncor		Open				Under Pine				Under Spruce			
			May	June	July	Aug	May	June	July	Aug	May	June	July	Aug
A	2.8	SSW	11.1	3.3	5.5	1.0	8.1	4.2	2.2	1.2	7.0	2.3	1.9	0.6
G	3.1	ESE	6.2	6.8	8.1	0.9	4.9	5.4	11.3	0.8	2.2	4.0	4.4	0.7
F	4.0	ESE	1.8	4.2	7.4	0.6	1.2	2.8	12.2	0.3	0.9	1.7	-	0.5
C	4.2	SSW	5.2	4.0	3.5	1.4	7.9	2.7	4.0	0.5	2.8	1.8	2.6	0.4
D	5.3	SSW	3.4	2.0	-	1.0	3.4	2.1	0.9	1.0	2.5	1.5	0.8	0.7
E	8.3	SSW	2.3	1.2	1.1	0.9	3.2	2.7	1.4	0.8	2.9	0.8	1.3	0.8
J	10.5	NW	0.3	1.3	1.5	1.4	0.9	1.4	1.8	1.3	0.7	1.3	0.9	0.9
K	23.7	NNW	0.4	0.3	1.2	1.5	0.5	0.5	0.9	1.0	0.1	0.1	0.7	0.9

Sulphur dioxide concentration appears to be highly dependent upon time of year, presumably as a result of changes in the prevailing wind direction. In general, the sites closest to the source had the greatest deposition at all times of year, and the two most distant sites represented background SO_2 levels for the area as a whole.

Sulphation plates placed at various heights in jack pine, black spruce, and aspen canopies substantiated the statement that the type of canopy is important to SO_2 deposition in forested areas (Figure 3). The open jack pine stand had a SO_2 deposition profile that would be expected in open areas with a sink of SO_2 at the ground surface. It mirrors the expected wind speed profile upon which it is at least partially dependent.

The other two profiles indicated the canopy structure. The black spruce stand became progressively more dense close to the surface whereas the aspen stand had a dense tree stratum separated from the high shrub stratum by about 3 m (3 to 6 m) of only the boles of the trees.

The importance of wind speed cannot be understated since it may be the primary factor causing the measured deposition profiles. Wind profiles are necessary before an assessment of the influence of the various plant species can be made. In spite of this limitation, it is apparent that SO_2 deposition to forested canopies of varying composition cannot be assumed to be the same.

3.1.2 Native Plants and Soils

Chemical analysis of selected plant species at sites A to E showed a substantial increase in S content at sites close to the pollution source (Table 2). Labrador tea and feathermoss had the highest S levels at all sites but the difference in S between sites A and E were similar for all species. Since there were substantial differences in S concentration between species at each site, no direct

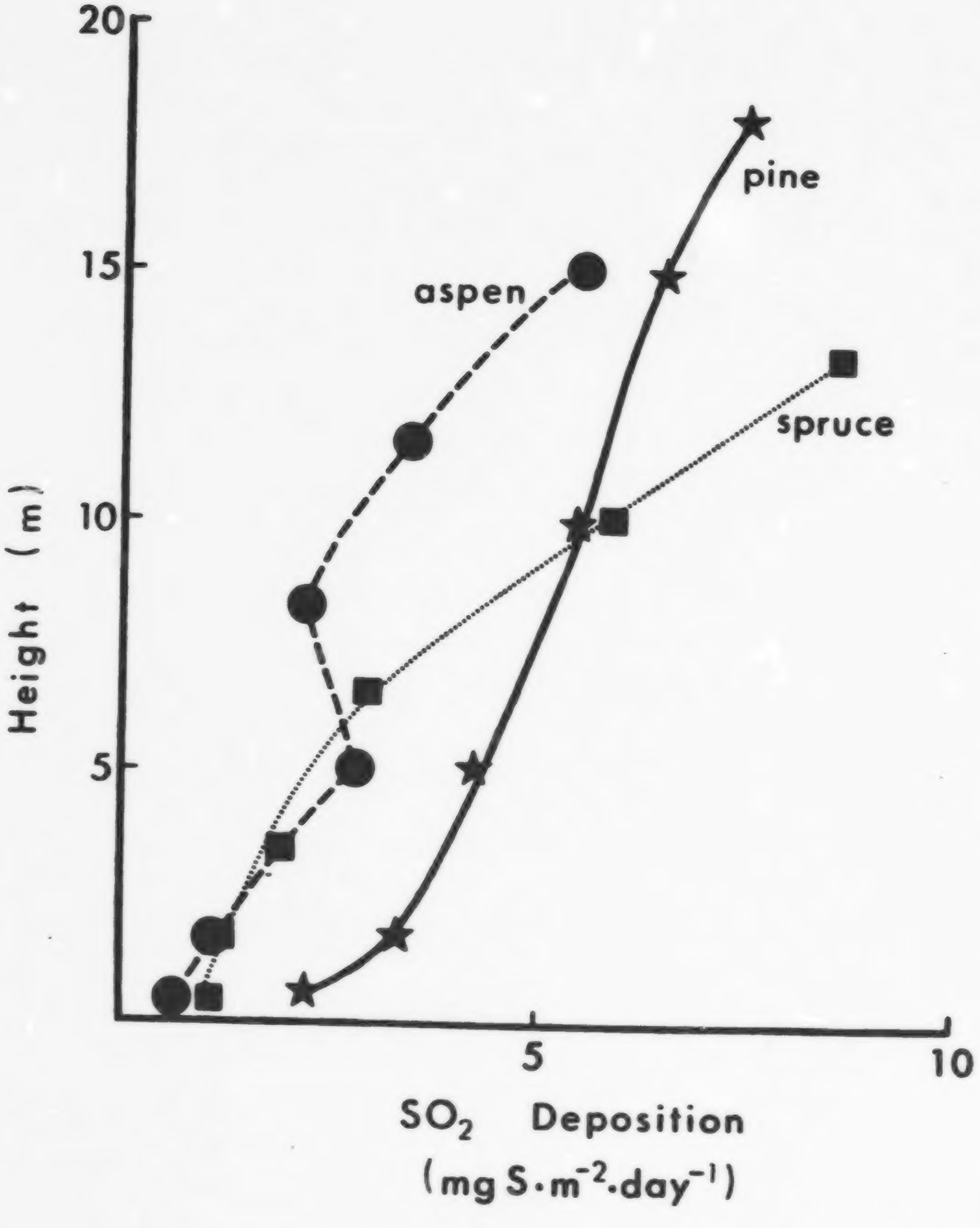

Figure 3. Profiles of SO_2 deposition to sulphation plates in three boreal forest stands in the Athabasca Oil Sands area. Values are averages of four measurements.

Table 2. Selected element content (ppm) of certain plant species at gradient sites in the vicinity of oil sands operations. Mean ± 95% confidence limits. Samples taken in 1977.

Site	Distance from Suncor (km)	Dry Phase			Moist Phase		
		Pinus banksiana	*Arctostaphylos uva-ursi*	Ground Lichens	*Picea glauca*	*Ledum groenlandicum*	Feather-moss
Sulphur							
A	2.8	1002 ± 48	796 ± 71	629 ± 38	918 ± 81	1409 ± 92	1289 ± 49
B	3.4	940 ± 60	601 ± 34	439 ± 51	718 ± 41	1288 ± 64	1434 ± 54
C	4.2	964 ± 28	677 ± 26	545 ± 27	735 ± 37	1146 ± 86	1419 ± 89
D	5.3	828 ± 30	559 ± 23	285 ± 25	691 ± 101	1180 ± 78	1195 ± 230
E	8.3	723 ± 40	423 ± 76	313 ± 74	496 ± 31	1054 ± 98	928 ± 89
Aluminum							
A	2.8	643 ± 60	397 ± 70	738 ± 369	303 ± 195	399 ± 54	2150 ± 1089
B	3.4	480 ± 83	549 ± 62	1051 ± 609	182 ± 46	473 ± 107	4772 ± 2162
C	4.2	626 ± 122	268 ± 54	620 ± 221	97 ± 31	203 ± 49	1294 ± 407
D	5.3	655 ± 100	196 ± 35	386 ± 181	253 ± 92	350 ± 76	2073 ± 262
E	8.3	668 ± 88	261 ± 54	392 ± 120	162 ± 21	276 ± 25	1528 ± 95
Iron							
A	2.8	191 ± 18	215 ± 31	293 ± 127	125 ± 34	248 ± 20	753 ± 373
B	3.4	199 ± 31	207 ± 32	417 ± 142	173 ± 26	475 ± 81	1093 ± 591
C	4.2	156 ± 30	162 ± 19	243 ± 73	102 ± 15	150 ± 24	715 ± 215
D	5.3	120 ± 25	190 ± 27	168 ± 61	300 ± 169	412 ± 78	2341 ± 338
E	8.3	331 ± 69	254 ± 49	241 ± 125	215 ± 31	338 ± 51	2165 ± 145
Phosphorus							
A	2.8	966 ± 129	1169 ± 145	382 ± 118	922 ± 90	1216 ± 94	494 ± 155
B	3.4	814 ± 105	848 ± 118	273 ± 86	1058 ± 102	1022 ± 79	754 ± 274
C	4.2	789 ± 80	960 ± 91	164 ± 41	919 ± 49	976 ± 125	620 ± 160
D	5.3	765 ± 48	821 ± 66	221 ± 41	763 ± 72	1061 ± 45	1060 ± 192
E	8.3	892 ± 19	958 ± 58	151 ± 30	1139 ± 80	1136 ± 113	1091 ± 56
Potassium							
A	2.8	3322 ± 432	4633 ± 231	489 ± 112	4199 ± 560	4063 ± 136	2093 ± 776
B	3.4	3186 ± 408	3616 ± 332	546 ± 227	3785 ± 430	3502 ± 236	3408 ± 1229
C	4.2	3556 ± 306	4691 ± 608	444 ± 122	3782 ± 228	4125 ± 509	2488 ± 714
D	5.3	3020 ± 429	4904 ± 77	398 ± 90	3011 ± 382	3988 ± 473	4111 ± 1228
E	8.3	3631 ± 289	4756 ± 359	321 ± 71	5115 ± 404	4439 ± 414	5675 ± 519

comparison could be made. Regressions were constructed for the S concentration of each species versus distance from Suncor Inc. and the Y intercept in each case was considered as maximum S content (100%). Regression coefficients were determined for relative S contents and this gave a measure of the decrease in S content with increasing distance.

Similar values were calculated for sulphation plates and for arboreal lichen S content (Table 3; Addison and Baker 1979).

Higher S levels close to the pollutant source did not appear to be related to soil S content and there was little difference among sites in the rhizophere (Ah, Ae, and Bm horizons; Table 4). The LFH horizon did show a distinct gradient in S either as a result of direct long-term deposition or because of addition by leaf-fall, or both.

Understory plant species in dry locations had a much greater increase in S with decreasing distance from the processing plant than did those related to the difference in stand density and the lushness of the lower stratum of the community. The dry sites were far more open than the moist ones and, hence, the pollutant could enter the stand and fumigate the ground vegetation much more readily. White spruce from moist sites on the other hand, had a greater rate of increase in S content than jack pine. In this case, both samples were collected well up the tree where SO_2 could easily impinge on the tissue. It is felt that the differences between these two species may be related to the resistance to entry of the gas imposed by the stomata.

3.2 MEASUREMENT OF RESPONSE

3.2.1 Growth and Reproduction of Jack Pine

3.2.1.1 Basal Area. There did not appear to be a relationship between the cross-sectional area increment and distance from the pollutant source (Table 5; Addison 1980b). There was actually greater growth in the last 10 years relative to the previous 10 years close to Suncor Inc. than at 10 km distance. Even though this form of self-standardization (comparing each tree's increment with itself before industrialization) can eliminate much of the site-induced variability, it appears that growth changes still need to be very large before they can be detected with any confidence.

Table 3. Regression coefficients of relative sulphur content versus distance from Suncor Inc. for selected plant species and sulphation plates (1977).

Material	Microsite Type	Microsite Location	Regression Coefficient
jack pine	dry	canopy	-4.5
bearberry	dry	ground	-6.5
ground lichens	dry	ground	-7.4
white spruce	moist	canopy	-6.3
labrador tea	moist	ground	-3.7
feathermoss	moist	ground	-5.1
sulphation plates	both	1.5 m	-8.2
arboreal lichens	dry	1.5 m	-9.6

Table 4. Total element content (ppm) of the primary soil horizons at the gradient sites in 1977. Values are averages of 2 to 4 samples.

Site	Distance from Suncor (km)	LFH	A Ah,Ae,Ahe	Bm	C
Sulphur					
A	2.8	634	207	35	87
B	3.4	-	91	15	5
C	4.2	498	35	9	9
D	5.3	128	108	44	33
E	8.3	68	13	25	33
Aluminum					
A	2.8	7300	8849	16364	11863
B	3.4	-	8223	18374	12434
C	4.2	7688	7348	10063	12841
D	5.3	4714	9773	18424	14271
E	8.3	6636	6709	14251	6401
Iron					
A	2.8	3718	3427	12240	7999
B	3.4	-	1875	8673	4157
C	4.2	2977	2530	6478	6422
D	5.3	1605	2473	13186	5686
E	8.3	2865	1983	11893	6326
Phosphorus					
A	2.8	956	246	339	111
B	3.4	-	417	565	297
C	4.2	878	320	623	310
D	5.3	541	102	262	93
E	8.3	1024	179	846	364
Potassium					
A	2.8	2162	4593	5892	5417
B	3.4	-	3571	6580	4703
C	4.2	2196	2661	2823	3932
D	5.3	1575	5337	5896	6387
E	8.3	1912	2627	4272	2996
Calcium					
A	2.8	732	261	147	142
B	3.4	-	216	105	83
C	4.2	543	113	77	103
D	5.3	199	192	124	147
E	8.3	571	80	120	85
Magnesium					
A	2.8	870	521	1024	764
B	3.4	-	414	1131	562
C	4.2	623	330	456	660
D	5.3	219	374	1140	749
E	8.3	300	259	868	467

Table 5. Ratio of wood cross-sectional area growth before versus after start-up of Suncor in the Athabasca Oil Sands area in 1966.

Site	Distance (km) and Direction from Suncor		Ratio of wood area 1968-77 : 1957-67 (mean ± 95% confidence limits)
A	2.8	SSW	2.16 ± 1.26
H	3.0	N	1.15 ± 0.16
G	3.5	ESE	1.02 ± 0.50
F	4.0	ESE	1.62 ± 0.29
J	10.5	NW	1.48 ± 0.29

3.2.1.2 Shoot Length. Over the past 5 years, the jack pine at Site A produced significantly ($p < 0.01$) longer leaders of the lateral branches than did jack pine at Site J (Table 6; Addison 1980b). This observation concurs with the basal area growth measurements that showed over twice the radial growth at Site A ($435\ mm^2 \cdot yr^{-1}$) than at Site J ($215\ mm^2 \cdot yr^{-1}$). The differences seen in Table 6 are presumably a result of substantially better growing conditions at Site A that have not been significantly affected by the presence of air pollutants.

3.2.1.3 Leaf Number. The needle number of the most recent three age classes indicated that jack pine sampled at Site A produced more needles than did those at Site J (Table 6; Addison 1980b). This is consistent with both the leader length and basal area measurements that also showed greater growth at Site A than at Site J. In years 3 and 4, however, there were fewer needles per age class at Site A (Table 7), although the differences were not significant. It is thought that the reduction in needle number in years 3 and 4 may be a result of premature aging and abscission of leaves caused by air pollutants. Because so many natural factors may influence needle retention, no definite conclusion is possible. It should be noted, however, that even if premature needle drop does occur in the vicinity of Suncor, there is no evidence that it detrimentally affects plant growth and survival.

3.2.1.4 Seed Germination. The jack pine seed germination rate appeared to be reduced close to Suncor operations (Table 8; Addison 1980b). Site A had significantly ($p < 0.05$) lower germination rate than Site E, whereas the other sites were intermediate and were not significantly different from either A or E. There was also a significant correlation of seed germination with distance from the source ($p < 0.05$). Seed germination rate did not appear to be related to tree age (Table 8).

Table 6. Leader length of lateral branches of jack pine at Site A and at Mildred camp (J) in the Athabasca Oil Sands area. (mean and 95% confidence limits)

Age Class	Distance between Bud Scale Scars (cm)	
	Site A	Mildred J
Current	21.2 ± 2.2	15.8 ± 1.4[a]
1 year	24.0 ± 2.5	19.3 ± 2.2
2 year	23.4 ± 2.2	17.5 ± 1.9
3 year	27.6 ± 2.5	17.9 ± 1.6
4 year	25.4 ± 2.9	21.2 ± 1.9

[a] There are significant ($p < 0.05$) differences between sites for each age class.

Table 7. Number of needles per age class on the lateral branches of jack pine at Site A and Mildred (J) in the Athabasca Oil Sands area (mean ± 95% confidence limits).

Age Class	Number of needles		
	Site A	Mildred J	Significance
Current	115 ± 10	104 ± 8	NS
1 year	119 ± 11	100 ± 9	0.05
2 year	132 ± 13	93 ± 8	0.001
3 year	58 ± 22	63 ± 13	NS
4 year	14 ± 10	22 ± 10	NS

Table 8. Jack pine seed viability of trees from the gradient sites in the Athabasca Oil Sands area.

Site	Distance (km) from Suncor	Tree Age (y)	Seed Germination (%)		
A	2.8	54.6	81.5[a]	│	
B	3.4	33.6	89.4	│	│
C	4.2	29.1	90.9	│	│
D	5.3	40.0	94.2	│	│
E	8.3	42.0	95.8		│

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[a] Lines join means that are not significantly different ($p < 0.05$) in a Student-Newman-Keuls test.

3.2.1.5 Biochemistry and Physiology. No significant differences in any of the metabolic responses measured were observed among the sites sampled. This may be owing to either the low concentrations of SO_2 experienced in these areas or the ability of spruce and pine to recover their metabolic functions between pollutant episodes. The concentrations of S in tissue (Table 2) were in the normal range for these species and it is felt that this, along with the plant's capability to detoxify the toxic species of sulphur (conversion of HSO_3^- and $SO_3^=$ to $SO_4^=$), that is responsible for the lack of response (Malhotra and Khan 1980). It should be pointed out that several of the biochemical and physiological responses measured here have been used to detect air pollution injury to plants near Flin Flon, Manitoba (Malhotra and Khan unpublished). This pollution source has been operating for a considerably longer period of time and releases both greater amounts of SO_2 and more persistent pollutants (heavy metals; particularly zinc) than oil sands operations.

3.2.2 Plant Community

The jack pine stands at the gradient sites (A to E) were selected because of their similarity. In spite of this, in 1977, there was considerable variability in species composition and cover of the lower stratum plants (Table 9). This variability did not appear to be related to either gaseous pollutant deposition (Table 1) or distance from Suncor Inc. The community structure and composition therefore, provided no evidence that the Suncor processing plant was having an effect on jack pine stands in the immediate vicinity of oil sands operations (i.e. < 8.3 km).

Measurements of the same sites in 1979 permitted direct comparison of each site with itself over a two year period. Changes in the stands could not be related to pollutants. It is thought that some of the changes in cover (Sorenson's index) at these sites were as a result of error in the placement of quadrats during repetitive

Table 9. **Association table of cover values of the vascular plants in the lower strata of jack pine stands (A to E) in the Athabasca Oil Sands area (July 1977).**

Species	Sites E	D	A	C	B
Trees					
Picea glauca	0.03				
Pinus banksiana	0.03		0.03		
Populus tremuloides	1.45	0.35	0.05		
Shrubs					
Alnus crispa		8.50	0.13	8.20	
Amelanchier alnifolia			0.08		0.45
Prunus virginiana			0.05	4.13	
Rosa acicularis			0.08		
Ledum groenlandicum	0.30	2.08			
Vaccinium myrtilloides	6.13	9.78	11.70	7.05	2.55
Ground Shrubs					
Arctostaphylos uva-ursi	0.85	2.05	12.95	14.15	27.40
Vaccinium vitis-idaea var. *minus*	2.93	10.48	1.23	1.98	2.45
Linnaea borealis var. *americana*		0.75	0.50	0.50	0.85
Herbs and Grasses					
Unidentified Grass	0.53	0.50	0.53	0.53	0.60
Lilium philadelphicum var. *andinum*		0.18			
Maianthemum canadense	0.55	0.98	0.95	0.28	0.50
Comandra pallida	0.03	0.05	0.60	0.58	0.50
Geocaulon lividum	0.03				
Potentilla tridentata		0.13	0.28		
Viola nephrophila			0.03		0.05
Epilobium angustifolium	0.03	0.13		0.35	0.33
Aralia nudicaulis				1.28	
Cornus canadensis	0.53	1.80	0.18		
Pyrola secunda					0.05
Apocynum androsaemifolumi				0.03	
Mertensia paniculata				0.03	
Melampyrum lineare	0.23	0.23	0.10	0.18	0.18
Campanula rotundifolia			0.03	0.03	
Solidago decumbens				0.03	0.10
Pteridophyte					
Lycopodium complanatum		0.03		0.98	2.70
Total Cover					
Vascular Plants	13.65	38.02	29.50	40.31	38.71
Lichens	76.90	36.55	50.28	50.63	33.03
Mosses				0.25	0.05
Stand Density (stems/ha)	100	175	150	75	400

sampling. Individual quadrats were mapped but not permanently marked and, hence, errors in repositioning may have been responsible for some of the changes seen in Table 10. In addition, the amount of natural change that goes on at these sites is unknown.

Table 10. Similarity in Sorenson's index between 1977 and 1979 sampling times of jack pine stands A to E .

Site	Distance from Suncor (km)	Percent Similarity
A	2.8	88.4
B	3.4	-
C	4.2	83.6
D	5.3	85.3
E	8.3	84.5

3.2.3 Studies of Naturally Occurring Lichens

3.2.3.1 Condition and Element Content. Detailed results and discussion of the survey of lichens in the vicinity of Suncor Inc. have been reported elsewhere (Addison and Puckett 1980). The conclusions from this study indicated that: (1) the pattern of element deposition measured by lichen thallus concentration appeared to be related to the distribution pattern of elements as measured by physical and chemical methods; (2) the accumulation of elements by *Evernia mesomorpha* and *Hypogymnia physodes* could be related to both gaseous and particulate emissions from industrial sources and to localized windblown dust components; (3) a single collection of lichen material can replace traditional physical monitoring methods in locations where only relative deposition estimates of pollutants are required. The use of lichens in this manner also permits a measure of biological response that can be partially related to the content of specific elements in the thallus.

3.2.3.2 Biochemical Condition. No pattern that could be related to pollutants was observed in any of the metabolic processes examined (Table 11). Only the healthiest material from each site was used in the analyses and, therefore, it appears that the lichens that do exist close to the pollutant source are metabolically active. It is felt that impact to lichens occurs on an event basis when environmental conditions and pollutant levels interact to damage parts of the thallus. This hypothesis would resolve the apparent inconsistency between the metabolic and the field survey and transplant studies.

3.2.4 Studies of Lichen Transplants

3.2.4.1 Community. Lichen community response to air pollutants characteristic of oil sands operations, has been demonstrated by

Table 11. Metabolic activity of *Evernia mesomorpha* from sites in the Athabasca Oil Sands area. Values are means of three replicates ± standard deviation.

Site	Distance from Suncor (km)	Acid Phosphatase units/g dry wt	^{14}C incorporation 10^5 cpm/g dry wt	Protein Biosynthesis 10^5 cmp/g dry wt
A	2.8	1.7 ± 0.2	70.9 ± 2.9	13.3 ± 1.5
C	4.2	3.1 ± 0.2	55.7 ± 4.1	22.9 ± 0.9
E	8.3	1.8 ± 0.3	62.6 ± 6.2	17.3 ± 3.6
J	10.5	-	-	15.0 ± 3.0
K	23.7	-	-	13.9 ± 3.3

Addison (1980b). Response of transplanted lichens in the immediate vicinity of Suncor appeared to be the result of a complex of factors that include several pollutants as well as natural environmental factors. The importance of several factors in lichen response was demonstrated by Addison and Puckett (1980). Since changes in lichen condition of transplants were consistent with changes occurring on non-transplanted branches in areas of low or negligible pollutant deposition, the concern that small changes in microenvironment may dramatically affect lichen community composition and cover on a short-term basis appears unfounded.

3.2.4.2 Element Content and Physiology. Available to total ratios of K and Mg in transplanted *Evernia mesomorpha* appeared to be unrelated to air pollution (Addison 1980b). Variability of all measurements was great and it is clear that, in its present form, the introduction of lichen material for detailed examination of sulphur uptake or changes in physiological condition is inadequate as a biomonitoring tool. The technique, however, may be effective if more meaningful biological measurements are made and greater replication is ensured.

4. BIOMONITORING APPROACH

To date, our approach has been to work with well-defined, permanently marked and well-documented sites. The advantages of this approach are as follows:

1. It permits simultaneous assessment of numerous factors that may respond to pollutants. This results in greater reliability as it substantially reduces the possibility of a chance occurrence being interpreted as a meaningful response.
2. It permits the interpretation of one factor through the use of others. For example, a discussion on the exposure of a target organism in a forest without a measure of stand type and density would be meaningless, as would a discussion of plant pollutant content without soils analyses and physical measurements of pollutant deposition.
3. It reduces the natural variability that has to be dealt with in all ecosystems by allowing measurements of the same site over time.
4. It permits the establishment of a system that takes into consideration different types and rates of response. Biomonitoring, if it is to be used as a method to provide early warning of pollutant injury to the forest, must use a combination of techniques of differing sensitivities with known relationships between them so that both questions, What has happened? and What will happen?, can be answered.
5. It permits comparison of different types of responses and allows testing of the reliability and reproducibility of specific techniques.

The approach, however, is not without disadvantages. Only a small number of sites (< 30) can be handled since the technique is labour intensive. Sites must also be representative of the area. This is a particularly difficult requirement to satisfy in heterogeneous areas. A team approach which is often difficult to maintain is essential owing to the multidisciplinary nature of the work.

In general, this approach when applied to the Athabasca Oil Sands area of Alberta met with mixed success. It was possible to demonstrate that pollutants characteristic of oil sands operations were not distributed evenly. Distinct gradients in soil LFH horizon, native plant element content, and sulphation plates were observed in the vicinity of Suncor Inc. It is expected that precipitation measures would also show the same pattern if it were not for the technical problems encountered.

Demonstration of plant response to the deposited pollutants was much more difficult. Long-term measures such as vascular plant community change, soil nutrient change, and tree growth could not be related to pollutant deposition. Some plant responses such as lichen condition and pollutant content, jack pine seed germination and lichen community change appeared to be influenced by Suncor, but the magnitude of response is not great enough to be of alarm. Physiological responses of vascular plants and lichens that were expected to be very sensitive were far too variable to be useful without further refinement.

Many other biomonitoring techniques in a wide variety of disciplines warrant examination. Support of both government and industry proposed for the next few years shall provide biological tools for the detection of airborne pollutant effects on terrestrial ecosystems.

5. REFERENCES CITED

Addison, P.A. 1980a. Baseline condition of jack pine biomonitoring plots in the Athabasca Oil Sands area, 1976-77. Prep. for the Alberta Oil Sands Environmental Research Program by Environment Canada, Canadian Forestry Service. AOSERP Report No. 98. 43 pp.

Addison, P.A. 1980b. Ecological bench-marking and biomonitoring for detection of airborne pollutant effects on vegetation and soils. Prep. for the Alberta Oil Sands Environmental Research Program by Environment Canada, Canadian Forestry Service. AOSERP Report No. 111. 48 pp.

Addison, P.A. and J. Baker. 1979. Interim report of ecological bench-marking and biomonitoring for detection of airborne pollutant effects on vegetation and soils 1975 to 1978. Prep. for the Alberta Oil Sands Environmental Research Program by Environment Canada, Canadian Forestry Service. AOSERP Report No. 46. 38 pp.

Addison, P.A. and K.J. Puckett. 1980. Deposition of atmospheric pollutants as measured by lichen element content in the Athabasca Oil Sands area. Can. J. Bot. 58: 2323-2334.

Baker, J. 1980. Differences in the composition of soils under open and canopy conditions at two sites close-in to the Great Canadian Oil Sands Operation, Fort McMurray, Alberta. Prep. for the Alberta Oil Sands Environmental Research Program by Environment Canada, Canadian Forestry Service. AOSERP Report No. 97. 24 pp.

Cain, S.A. and G.M. de O. Castro. 1959. Manual of vegetation analyses. Harper and Row Inc., New York, N.Y. 422 pp.

Carson, J.A., J.M. Crepin, and P. Nemunis Siugzdines. 1972. A sulphate-sulphur method used to delineate the sulphur status in soils. Can. J. Soil Sci. 52: 278-281.

Chan, C.C.Y. 1975. Determination of total sulphur in vegetation by a turbidimetric method following oxygen-flask combustion. Analy. Lett. 8: 655-663.

Clough, W.S. 1974. The deposition of particles on moss and grass surfaces. Environmental and Medical Sciences Division, U.K.A.E.A. Research Group, Atomic Energy Research Establishment, Harwell. 20 pp.

Douglas, G.W. and A.C. Skorepa. 1976. Monitoring air quality with lichen: a feasibility study. Syncrude Canada Ltd. Environmental Research Monograph 1976. 269 pp.

Hilchie, G.J. and J.K. Ryan. 1980. Approaches to the design of a biomonitoring program using arthropods as bioindicators for the AOSERP study area. Prep. for the Alberta Oil Sands Environmental Research Program by McCourt Management. AOSERP Report No. 105. 47 pp.

Malhotra, S.S. and A.A. Khan. 1979. Interim report of physiology and mechanisms of airborne pollutant injury to vegetation 1978-79. Prep. for the Alberta Oil Sands Environmental Research Program by Environment Canada, Canadian Forestry Service. AOSERP O.F. 2. 23 pp.

Malhotra, S.S. and A.A. Khan. 1980. Physiology and mechanisms of airborne pollutant injury to vegetation 1979-80. Prep. for the Alberta Oil Sands Environmental Research Program by Environment Canada, Canadian Forestry Service. AOSERP Report No. 110. 49 pp.

Richardson, D.H.S. and E. Nieboer. 1981. Lichens and pollution monitoring. Endeavor 5: 127-133.

Takyi, S.K. and M. Nyborg. 1977. Sulphur dioxide and acidification of soils in the oil sands area. Pages 1-50. in: Soil research related to revegetation of the oil sands area. Prep. for the Alberta Oil Sands Environmental Research Program by Alberta Forest Service. 50 pp.

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SYNERGISMS OF CONTAMINANT INTERACTION

by

P.A. ADDISON

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"Synergisms of Contaminant Interaction"

P.A. Addison

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SYNERGISMS OF CONTAMINANT INTERACTION

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INTRODUCTION

In the natural environment, pollutants rarely if ever occur singly and never affect either a plant or soil without some external influence of the natural environment. In spite of this somewhat obvious observation, researchers and managers alike have proceeded to ignore the importance of other factors including other pollutants when setting air quality criteria. The result of this is, that in many cases, emission standards either do not provide adequate protection for the environment, or place unrealistic restrictions on industry.

In the early 1950's, several observations indicated that pollutants in combination could be much more damaging to crop plants than when they occurred singly (Thomas et al. 1952). The earliest work was done with SO_2 and O_3 and using foliar symptoms of injury. In the early 1970's and throughout that decade, renewed interest in the effect of mixed pollutants resulted in a very large number of plants being tested as to their sensitivity to pollutant mixtures. Work was no longer confined to SO_2 and O_3 but included studies on NO_2 and SO_2, SO_2 and HF, and SO_2 and peroxyacetyl nitrate (PAN). With all combinations, measurements no longer required the development of visible symptoms owing to the development of more sensitive techniques such as biochemical, physiological, biomass or yield responses. The tools now developed could examine the influence of pollutant mixtures well before or without visible symptom development. The 1980's promise continued work on mixed pollutants and several laboratories are currently working with three or more pollutants (ie. SO_2, NO_2 and O_3). It is safe to say that in almost all cases, emphasis has been placed on crop plants owing to their economic importance and their proximity to the major emission sources in North America and Europe. The bulk of the studies on pollutant mixtures has been done in the laboratory using pollutant levels that have been recorded in the field. Much work is still required before this laboratory information can be interpreted relative to field situations.

We often hear the terms synergistic, antagonistic and additive related to the magnitude on plant response to mixtures of pollutants. In order to prevent any confusion, a definition of terms is required.

ADDITIVE Plant response to a pollutant mixture is the same as would be expected from the concentration of each pollutant presented singly.

LESS THAN ADDITIVE Plant response to a pollutant mixture is less than would be expected if each pollutant was presented singly.

GREATER THAN ADDITIVE Plant response to a mixture of pollutants is greater than would be expected if each pollutant was presented singly.

In addition, several terms describe how the pollutants in a mixture are presented to the organism of interest.

SIMULTANEOUS The pollutants are presented as a true mixture with all present at all times.

SEQUENTIAL Pollutants are presented one after another in some defined order.

INTERMITTENT Periods without pollutants alternate with fumigations

The approach of this workshop to try to determine what the environment is capable of assimilating is the only way in which maximum emissions can be combined with adequate environmental protection. I would suggest that in small quantities, all naturally occurring substances can be assimilated. The amount that can be incorporated without significant modifications of the system depends upon both the nature of the element or compound and its concentration (Table 1). Of course, the definition of what significant means is an essential prerequisite to the determination of assimilatory capacity.

TABLE 1. Examples of materials with differing capabilities for assimilation into an ecosystem.

Pollutants assimilated at moderate levels	Pollutants assimilated at very low levels or not at all
SO_2	Heavy Metals
NO_2	Radionuclides
O_3	Pesticides (DDT etc.)
H_2S	

The first set of componds which fortunately represent the majority of pollutants from forest industries can be incorporated into existing nutrient cycles and if not at too high a level or for too long, will be totally assimilated. The second set of compounds tend to accumulate in parts of the system owing to exceptionally long turn-over rates. In this second case, it would be more useful to use the term "carrying capacity" rater than "assimilatory capacity".

In dealing with the effect of mixtures of pollutants, a whole series of factors must be considered over and above those considered with single pollutants. The ratio of concentrations, how the pollutants are presented, the mechanism of pollutant uptake, mode of action of the pollutants,etc. must all be sorted out. Add to that the complexity of the natural environmental interactions and interdependencies and one ends up with an exceptionally complex situation. The list of factors is virtually unlimited (see Table 2) and explains why almost all of the mixed pollutant work has been carried out in the laboratory where environment and pollutants can be controlled to some extent.

Table 2. Partial list of factors that influence the response of plants to air pollutants.

Atmospheric Factors
- Light quality, quantity and photoperiod
- Temperature
- Humidity
- CO_2 concentration
- Wind speed
- Precipitation

Other Factors
- Fire
- Insects
- Disease
- Other plants
- Animals
- Man

Plant Factors
- Genotype
- Phenotype
- Phenological stage
- Time of year
- Age

Soil Factors
- Temperature
- Moisture
- Aeration
- Drainage
- Nutrition
- Topography
- Parent material
- Soil organisms

OVERVIEW

1. Interaction of Gaseous Pollutants

By far the greatest amount of information in the literature on pollutant mixtures deals with combinations of gases. Most work has been on the interaction of SO_2 and O_3 on the response of crop plants. The result of these studies are variable and appear to be highly dependent upon plant species. Responses reported include greater than, less than and additive (Reinhart et al. 1975). Combination of SO_2 and O_3 are estimated to do more damage to woody plants than all other pollutants together (Davis and Gerhold 1976) but, in spite of this, only a very little work has been done on native tree species. Several urban (Karnosky 1980) and Eastern Deciduous Forest (Carlson 1979) tree species have been studied but aspen is the only wide ranging tree species whose response to $SO_2 + O_3$ has been examined (Karnosky 1976). Some field and laboratory work has been done on the response of eastern white pine to $SO_2 + O_3$ (Dochinger et al. 1970).

Combinations of NO_2 and SO_2 may actually be of greater interest in the case of forest industries since both are generated from several of the processes. This combination results in a response in agricultural species that is at least additive. There are numerous examples of cases where the response in greater than additive in both agricultural crops (Bennet et al. 1975) and forest species (Malhotra and Khan 1980). As with $SO_2 + O_3$ combinations, much of the work has relied on the development of visible symptoms but the application of biochemical and physiological tools to these studies has enhanced our capability to determine early responses (Beckerman and Hofstra 1980, Malhotra and Khan 1980). Numerous other gas mixtures have been examined with a wide variety of responses of agricultural crops examined (Table 3).

Table 3. Visible symptom response to mixtures of gaseous pollutants.

Mixture	Response
$SO_2 + O_3$	+, 0, -
$SO_2 + NO_2$	+, 0
$NO_2 + NO$	+
$SO_2 + HF$	+, 0, -
PAN + SO_2	+
$O_3 + NO_2$	-
O_3 + PAN	+, 0, -
$O_3 + HF$	undefined
$NO_2 + SO_2 + CO_2$	-
$NO_2 + SO_2 + O_3$	+
$NO_2 + SO_2 + O_3$ + misting	+

2. Interaction of Soil Constituents

Interaction among soil constituents is both well known and well documented, particularly in the field of soil fertility. Ratios of C, N, S, and P have been examined in detail and, in many cases, an optimum ratio of these elements for the growth and yeild of crop plants has been determined. Excessive amounts of elements also interact resulting in a whole series of effects such as changes in the soil flora or fauna, changes in available nutrients and changes in basic soil processes such as decomposition and nitrification. The influence of H^+ concentration or pH on both terrestrial and aquatic systems has recently received much attention owing to the concern in eastern North America and Europe of acid rain. In addition to direct acidity effects, lower pH tends to increase the solubility of most metals. Increased solubility of metals in the soil system can have several effects which include 1) complexing of essential plant nutrients such as P and S as is the case for Al and Fe, 2) being toxic to root growth (ie. Cu, Ni, Cd etc.), 3) competing with required elements in plant nutrient uptake (S versus Se, K versus Na).

In addition to the amount of an element present in the soil system, the form that that element has is exceptionally important in determining its influence on the plant-soil system. The chemical form of an element will influence solubility which as mentioned above, is important from the point of view of leaching, plant availability and toxicity. It has been demonstrated that heavy metals associated with certain organic compounds may be both more soluble and more toxic to plants. The reverse may also occur.

In general, the below-ground system is very poorly understood even without the presence of pollutants at all. A large number of physical factors such as drainage, texture, porocity (also see Table 2) all can influence how pollutants will affect soil chemistry. This complexity means that the influence of pollutant mixtures on the ecosystem defies prediction at this time.

3. Interaction of Gaseous Pollutants with Soil Constituents

The influence of soil factors on the way in which gaseous pollutants influence plants is very poorly understood. Very little work has been attempted. It has been shown, however, that nutrient status influences the way in which plants such as peas are affected by SO_2 (Jager and Klein 1976). There is also some evidence that absorbed atmospheric S may compensate for sulphur deficient soils in plant nutrition. The influence of soil cantamination seems to be detrimental and Cd among other heavy metals has been shown to enhance the phytotoxicity of O_3 in pea seedlings and that of SO_2 in maple (Ormrod 1977, Lamoreaux and Chaney 1978). In our own work (Addison et al. in preparation), we found that Boreal Forest Conifers grown in tailings sand from Oil Sand Extraction Operations were twice as sensitive to SO_2 as those grown in their native soil.

In general, so few studies have been completed that no real pattern can be described. Soil contamination can influence plant metabolism which in turn dictates the response of the plant to gaseous pollutants. Whether a combination of a soil and a gaseous pollutant is greater than, less than or additive, depends upon too many factors to make generalizations.

FACTORS MODERATING POLLUTANT RESPONSE

1. Pollutant Form

The form or forms of the various pollutants characteristic of forest industries will be dealt with by others. In dealing with the topic of pollutant mixtures, the form of the pollutants, their ratio and the ultimate concentrations are all important inputs in the prediction of plant response. Changes in pollutant form in the atmosphere have been addressed, but with numerous pollutants and pollutant combinations, the changes that occur after deposition may be far more important to organisms. The mechanisms by which these transformations occur vary greatly and may require the action of soil organisms. Sulphur oxidizing bacteria are required to transform elemental S to SO_4 and in most coniferous forest litter, fungi are largely responsible for decomposition which transforms organic S to SO_4 and produces soluble organic compounds which influence the solubility and toxicity of heavy metals. Chemical reactions also are important as evidenced by the influence of pH on soil chemistry as mentioned above. A detailed understanding of soil chemical and biological processes is required before pollutant form in the soil and its influence on plants can be predicted.

2. Climate and Time of Year

As with the section above, most of the factors that influence pollutant uptake and plant response will be discussed by others. The influence of the natural environment is exceptionally important and may be even more critical and complicated when dealing with mixed pollutants. This is particularly apparent when the mechanism of pollutant uptake differs among pollutants in the mixture (ie. a soil versus a gaseous pollutant). The influence of the environment may be very complex. For example, a well known response of plants to heavy metals is the reduction of root growth. In situations when water is limiting, reduced root growth may result in severe plant water deficit and stomatal closure. This in turn may reduce pollutant gas uptake and protect the plant. On the other hand, gaseous pollutants such as SO_2 may cause the stomata to open and with the roots not able to absorbe adequate moisture owing to reduced growth, the plant may dehydrate. Soil temperature and nutrient content, air temperature and humidity, wind speed, light, stage of development and the time of year may all influence the way in which the response of a plant to soil water is affected by these two pollutants.

3. Soil and Plant Chemistry

Many of the soil and plant chemistry considerations that are important in pollutant interactions have been discussed above. It has been shown that soil nutrition influences the impact of SO_2 on peas (Jager and Klein 1976) and that conifers growing on tailings sand are predisposed to injury by SO_2 fumigation.

Soil factors such as the buffering capacity dictate how acid deposition will influence the soil-plant system. The impact of acid deposition in turn, is controlied to some extent by what is already present in the soil (ie. metals may become soluble and toxic or essential plant macroelements may be leached from the soil).

Plant chemical differences also influence the extent of injury from pollutants. The differences among the sensitivities of plants appear to be largely related to the capability of the plant's chemistry or metabolism to either exclude the pollutant or to detoxify pollutants such as dissolved SO_2 or NO_2 or their mixture.

4. Soil and Plant Physics

Physical concerns are related to the deposition, entry and retention of pollutants either singly or in combination in the system. Factors such as the canopy type, density and height, stomatal resistance, moisture status etc. all affect the absorption of gaseous pollutants and the deposition patern of particulate or aqueous pollutants. These factors are largely independent of what the pollutants are but are dependent upon the physical characteristics (eg. particle size, state or reactivity with surfaces). Retention of pollutants is also influenced by physical factors such as soil texture, porosity etc. which control drainage.

MODE OF ACTION OF POLLUTANT INTERACTION

Perhaps the best way to indicate how mixtures of pollutants influence an ecosystem is to cite case histories of the overall effects resulting from pollutant combinations. Sudbury is possibly better known as a pollution source than it is as a nickel-copper producer. For years SO_2 was blamed for the virtual elimination of vegetation in the area. It is now apparent that the vegetation damage so obvious in the Sudbury area was not the result of SO_2 alone but was caused by a series of events relating to the impact of both SO_2 and heavy metals. Exposure of plants to SO_2 causes tissue to age much more rapidly than it does in clean air. In addition, bacteria and fungi which are responsible for decomposition of organic matter, appear to be particularly sensitive to SO_2. In the Sudbury area, it appears that these two responses have resulted in a large increase in the amount of dead material on the soil surface (Freedman and Hutchinson 1980). The standing dead material acts as fuel for forest fires and Sudbury has had a history of frequent forest fires. Once plant and litter cover was removed, heavy metals such as Cu and Ni penetrated to the mineral soil and produced a substrate that would no longer support plant life except in a few isolated pockets. Without the mantle of organic material on the soil surface, erosional forces created the surface characteristics we see today.

Sudbury is not the only place where a combination of pollutants rather than a single pollutant has caused the effects on the ecosystem.

In western Montana, damage to a ponderosa pine forest near a pulp and paper mill was attributed to a combination of SO_2 and reduced sulphur compounds. In the Athabasca Oil Sands area, impact on lichens was accounted for by a combination of S and Vanadium from the source and Al and Titanium from surface disturbance. Although numerous studies have attempted to relate forest damage to single pollutants, in most cases, it appears that a combination is the ultimate cause. This can be the case even when there is only one element involved such as S in the case of sour gas plants. Combinations of SO_2 and elemental S appear to be far more damaging than SO_2 alone.

SUMMARY

In summary, we do know alot about the influence of mixed gaseous pollutants on the physiology and visible symptom development of crop plants. Much less is known of the interaction of soil and gaseous pollutants or how pollutants change in soils. The information that is available can be utilized effectively as can the great wealth of information on the response of plants to their environment in order to estimate the ultimate impact of pollutant combinations on an ecosystem. This however, cannot be accomplished without detailed studies on how forest species compare with crop plants with respect to mixed pollutant effects and without detailed information on the state of the environment and the major factors affecting ecosystem processes in the area of concern. Literature studies will provide much to the understanding of how pollutants both singly and in combination could influence forest systems but they cannot replace well designed and well carried out multidisciplinary research on the influence pollutants characteristic of forest industries on the forest ecosystem. Descriptive work so prevalent in the literature is an important first step but without a substantial effort on how ecosystem processes are affected by pollutants, the objective to determine the assimilative capacity of forest systems for pollutants is not possible.

LITERATURE CITED

Addison, P.A. (in preparation) Sensitivity of boreal forest woody plants to SO_2 and the effect of a contaminated substrate.

Beckerson, D.W. and G. Hofstra. 1980. Effects of sulphur dioxide and ozone, singly or in combination, on membrane permeability. Can. J. Bot. 58: 451-457.

Bennett, J.H., A.C. Hill, Abbas Soleimani and W.H. Edwards. 1975. Acute effects of combination of sulphur dioxide and nitrogen dioxide on plants. Environ. Pollut. 9: 127-132.

Carlson, R.W. 1979. Reduction in the photosynthetic rate of *Acer*, *Quercus* and *Fraxinus* species caused by sulphur dioxide and ozone. Environ. Pollut. 18: 159-170.

Davis, D.D. and H.B. Gerhold. 1976. Selection of trees for tolerance to air pollutants. Proc. Better Trees for Metropolitan Landscapes Symp. USDA Forest Service Gen. Tech. Rep. NE-22. pp 61-66.

Dochinger, L.S., F.W. Bender, F.O. Fox and W.W. Heck. 1970. Chlorotic dwarf of eastern white pine caused by ozone and sulphur dioxide interaction. Nature 225: 476.

Freedman, B. and T. C. Hutchinson. 1980. Effects of smelter pollutants on forest leaf litter decomposition near a nickel-copper smelter at Sudbury, Ontario. Can. J. Bot. 58: 1722-1736.

Jager, Von H.J. and H. Klein. 1976. Modellversuche zum Einfluss der Nahrstaffversorgung auf die SO_2-Empfindlichkeit vin Planzen. Eur. J. For. Path. 6: 347-354.

Karnosky, D.F. 1976. Threshold levels for foliar injury to *Populus tremuloides* by sulphur dioxide and ozone. Can. J. For. Res. 6: 166-169.

Karnosky, D.F. 1980. Chamber and field evaluations of air pollution tolerances of urban trees. Presented to the 56th annual meeting of the International Society of Arboriculture in Hartford, Conn on August 13, 1980. pp 99-105.

Lamoreaux, R.L. and W.R. Chaney. 1978. Photosynthesis and transpiration of excised silver maple leaves exposed to cadmium and sulphur dioxide. Environ. Pollut. 17: 259-268.

Malhotra, S.S. and A.A. Khan. 1980. Physiology and mechanisms of air-borne pollutant injury to vegetation 1979-80. Prep. for the Alberta Oil Sands Environmental Research Program by Environment Canada, Canadian Forestry Service. AOSERP Report No. 110. 23 pp.

Ormrod, D.P. 1977. Cadmium and nickel effects on growth and ozone sensitiviy of pea. Water, Air and Soil Pollut. 8: 263-270.

Reinert, R.A., A.S. Heagle and W.W. Heck. 1975. Plant responses to pollutant combinations. IN: Mudd, J.B. and T.T. Kozlowski (eds.) Responses of Plants to Air Pollution Academic Press Inc., New York. pp 159-177.

Thomas, M.D., R.H. Hendricks and G.R. Hill. 1952. In: McCabe, L.C. (ed.) Air Pollution, McCraw - Hill, New York. pp 41-47.

PUBLICATIONS IN REVIEW

SULFUR DIOXIDE SENSITIVITY OF WOODY BOREAL FOREST SPECIES GROWN ON NATIVE SOILS AND TAILINGS

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Sulfur Dioxide Sensitivity of Woody Boreal Forest Species Grown on Native Soils and Tailings[1]

P.A. ADDISON, S.S. MALHOTRA, AND A.A. KHAN[2]

ABSTRACT

Sulfur dioxide sensitivity was tested for several boreal forest woody species growing in a Degraded Dystric Brunisol and in oil sands tailings. The ranking of species sensitivity to air pollutants is a prerequisite for the interpretation of air pollution injury to vegetation in the field and for the selection of species for revegetation of mined oil sands. Fumigation with 0.34 ppm SO_2 significantly reduced the net assimilation rate (NAR) of CO_2 in all species and produced visible symptoms of injury. The response of NAR in deciduous species was significantly more rapid than in conifers or in an evergreen angiosperm. Visible symptoms also appeared earlier in deciduous species but in all cases did not appear until NAR had decreased considerably. The response of these previsible and visible injuries appeared to be related to differences in sulfur uptake.

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Conifers growing in oil sands tailings responded to SO_2 with a significantly more rapid decrease in NAR as compared with these growing in the Brunisol. Sulfur uptake and visible symptom development were not different on the Brunisol as compared with tailings sand.

Additional Index Words: photosynthesis, sulfur uptake, visible symptoms.

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INTRODUCTION

In the Athabasca Oil Sands area of northeast Alberta, Canada, extraction of petroleum deposits has resulted in emissions of SO_2 and other pollutants into the airshed (Shelfentook, 1978). Little obvious forest damage has occurred so far (Addison and Baker, 1979), and only lichens have demonstrated any response that could be related to air pollution (Addison and Puckett, 1980). The potential impact of industrial emissions on the boreal forest in this area, however, could be substantial as a result of both current and proposed development.

Determination of species sensitivity to air pollutants is a prerequisite for the interpretation of air pollutant injury to vegetation and changes in species composition or cover in the field. The sensitivity of woody boreal forest species to SO_2 has been determined from visible symptoms both in the field (Dreisinger, 1965) and under experimental conditions (Keller and

Bucher, 1976). A number of reports have described visible (Jacobson and Hill, 1970; Malhotra and Blauel, 1980) and previsible (Keller, 1980; Malhotra and Khan, 1982) pollutant injury of various plants. The concept that each plant species has a threshold level for SO_2 injury was formulated many years ago (O'Gara, 1922), but the complexity of natural environmental factors has limited its applicability in field situations (Mudd, 1975). Both external (climatic and edaphic) and internal (resistance and stage of development) growth factors have been shown to affect plant response to various pollutants, including SO_2 (Guderian, 1977). In the case of SO_2, several environmental factors that influence pollutant gas uptake have a distinct effect on plant response (Black and Unsworth, 1979; Majernik and Mansfield, 1972). The present study was designed to determine SO_2 sensitivities of dominant tree and shrub species from the Athabasca Oil Sands area and to examine the effect of SO_2 on the photosynthetic response of conifers grown in oil sands tailings.

MATERIALS AND METHODS

Collection and Growth of Plants

Representative dominant woody species of the oil sands area were collected in their native soil (Degraded Dystric Brunisol) at a site about 2 km north of Fort MacKay (Fig. 1). The species (3 to 8 years old) included Populus tremuloides Michx. (aspen), Salix sp. (willow), Alnus crispa (Ait.) Parsh (green alder), Betula papyrifera Marsh (paper birch), Picea glauca (Moench) Voss (white spruce), Picea mariana (Mill.) BSP (black spruce), Pinus banksiana Lamb. (jack pine), and Ledum groenlandicum Oeder (labrador tea). Deposition of SO_2 was at background levels in this area (Addison, 1980).

Picea glauca, P. mariana, and Pinus banksiana were also collected from the tailings dike of the Suncor Inc. plant 30 km north of Fort McMurray (Fig. 1). This dike is located in the zone of maximum air pollution impingement (Barrie and Whelpdale, 1978). These species had been about 1 year old when planted on the dike and were 5-7 years old when studied.

All plants were potted in the soil in which they had been growing in the field and were grown under greenhouse conditions at 20°C, 50% relative humidity, and with supplemental cool white fluorescent light (18 h photoperiod) for about 2 months before being transferred to a controlled environment chamber for experimentation. In the controlled environment chamber, an 18 h photoperiod was maintained by a combination of high pressure sodium, metal halide, and tungsten filament lamps. Light intensity was programmed to simulate diurnal variations, with a maximum photon flux density of 500 μmol $m^{-2}s^{-1}$. Temperature was held constant at 18°C, and dew point was also constant at 15°C. Plants were watered three times a week and were fertilized weekly with quarter-strength Hoagland's solution (#2).

Fumigation Conditions

The actively growing plants were held in the controlled environment chamber in clean air for 10 to 25 days to determine prefumigation net assimilation rates. The plants then were fumigated with 0.34 ppm SO_2 (Canadian ambient air quality standard for 1 h) continuously to the end of the experiment. Pure SO_2 was injected into an intake duct by a motor-driven needle valve, and SO_2 flow was automatically adjusted by a feedback control mechanism to supply the desired concentration (± 2%) within the chamber. The

SO_2 concentration was continuously monitored with a Monitor Labs SO_2 analyzer.

Net CO_2 Assimilation Rate

Net CO_2 assimilation and dark respiration rates were measured in a controlled environment cuvette at 18°C with a 15°C dew point. Ambient air from outside the building (300-330 ppm CO_2) was mixed in two 200-L drums to provide uniform CO_2 concentrations. An open gas-flow system with a Beckman Model 865 infrared CO_2 analyzer was used to measure the difference between the CO_2 concentrations of the cuvette airstream and the reference stream.

During CO_2 assimilation measurements (which were done every 2-7 days), plants were maintained under 600 μmol $m^{-2}s^{-1}$ photon flux density (high pressure sodium and metal halide lamps). Dark respiration was measured with the cuvette completely covered with black plastic. The CO_2 analyzer was calibrated twice daily with precalibrated gas cylinders. An average of five replicates was used for each species growing on native soil, and an average of three replicates was used for those on tailings sand.

Gas exchange rates during fumigation with SO_2 were expressed as a percentage of the rate before the pollutant gas was added. An arc sine transformation was carried out on these proportional values to ensure equality of variances for statistical purposes. A linear regression was then calculated for both the prefumigation and fumigation conditions of each species. The regression coefficients (slope of the regression line) were compared with zero slope and with each other in order to determine if there was a response by the species to SO_2 and to rank the species in order of sensitivity to SO_2.

Visible Symptoms

A photographic record of the visible symptoms of SO_2 toxicity of all species was maintained during the experiments. Photographs were taken daily during the clean air period in the growth chamber and every 2 to 7 days after the SO_2 fumigation had begun.

Sulfur Analysis

Sulfur content of each species was determined by using an oxygen flask combustion technique (Chan, 1975) to oxidize plant tissue samples collected both before and after fumigation. Sulfur was determined with a modified Johnson-Nishita technique (Carson et al., 1972).

RESULTS AND DISCUSSION

All plant species collected from the Fort McKay site showed normal net assimilation rates (NAR) in clean air (Sestak et al., 1971; Table 1). The NAR of the two Picea species growing in tailings material was also normal in clean air; however, the NAR of Pinus banksiana was significantly lower ($p < 0.05$) in tailings-grown plants than in the Brunisol-grown plants (Table 1). The prefumigation regression coefficients of NAR versus time (Table 2) also showed that none of the plants was under stress, and there was no significant change in NAR with time while the plants were in clean air. The only exception was Pinus banksiana, which showed a significant increase ($p < 0.05$) in NAR with time, apparently as a result of increased photosynthetic leaf area because of growth. In general, before the plants were exposed to SO_2 they were metabolically active and showed no adverse effects from either the environmental conditions in the growth chamber or the measurement procedure.

All plant species growing in the Brunisol showed a significant reduction in NAR when they were exposed to 0.34 ppm of SO_2 (Table 2), though the rate of decrease with time varied greatly among species, as indicated by the fumigation slope (Table 2). The deciduous species had greater rates of reduction in NAR than did the evergreen species. The fumigation slopes of NAR were significantly more negative (Simultaneous Test Procedure; $p < 0.05$) for Salix sp., Betula papyrifera, and Populus tremuloides than for any of the evergreen species. The large variability in the measurement of NAR of Alnus crispa made classification difficult, but it was decided to include this species with the other deciduous species because of its sulfur uptake and the time required for the onset of visible symptoms of SO_2 injury (Table 2). Ledum groenlandicum and the three conifer species, Picea glauca, P. mariana, and Pinus banksiana, had the lowest rates of reduction of NAR when exposed to SO_2. L. groenlandicum was by far the most resistant angiosperm (Table 2) and appeared to be as sensitive as the most sensitive conifer on the Brunisol. Pinus banksiana appeared to be the most resistant conifer to SO_2, but the difference between prefumigation and fumigation slopes for this species was greater than that for either of the Picea species, suggesting that it is actually more sensitive.

There was very little effect of SO_2 fumigation on dark respiration and no pattern of response was observed for any of the species. This observation is consistent with agricultural species for which SO_2 fumigation stimulates or produces no effect on dark respiration (Black and Unsworth, 1979; Furukawa et al., 1980).

Both visible symptom development and the change in NAR with time in response to SO_2 were similar for the plants grown in the Brunisol. The time required for development of visible symptoms was significantly longer ($p < 0.05$) in the evergreens than in the deciduous species (Table 2). There were no significant differences among the species within each group. Although the sensitivity to SO_2 indicated by visible symptoms and NAR followed similar trends, changes in NAR were observed well before visible symptoms were evident. A reduction in NAR and other physiological processes in response to SO_2 before the development of visible symptoms has been observed under field conditions (Legge et al., 1978) and in the laboratory (Malhotra and Khan, 1982).

Sulfur (S) uptake (combination of soil and atmospheric) was variable among species (Table 2). *Populus tremuloides* S uptake was not significantly different ($p > 0.05$) from the uptake of the evergreen species, whereas *Betula papyrifera*, *Alnus crispa*, and *Salix* sp. all had significantly greater ($p < 0.05$) S uptakes. Sulfur uptake was significantly correlated with the fumigation slope of NAR ($r = 0.76$; $p < 0.05$), indicating that there may be a relationship between the uptake of S and the response of the species as a whole.

In general, species sensitivity to SO_2 has been found to be controlled by both the ability of the plant to exclude pollutants and its capability to convert absorbed SO_2 into nontoxic forms (Malhotra and Khan, 1982). Coniferous trees have higher stomatal resistances (40-80 s cm^{-1}) than angiosperm woody plants (5-10 s cm^{-1}) (Gates, 1966), which would explain the differences between deciduous and coniferous species groups in Table 2. The differences in NAR among species within each group, on the other hand, may be related to the tolerance of certain species to the pollutant gas. Fumigation studies on Glycine max L. have shown that the conversion of toxic forms of dissolved SO_2 (SO_3^{2-}) was more rapid in resistant cultivars than in sensitive ones (Miller and Xerikos, 1979). The order of SO_2 sensitivity of the species tested in this study is shown in Table 2, except that Pinus banksiana was classed as being more sensitive than either of the Picea species in the Brunisol because of its significant and positive prefumigation NAR slope.

Sensitivity of the conifer species appeared to be substantially increased when they were grown in tailings sand. As with conifers grown in their native soil, there was a significant reduction ($p < 0.05$) in NAR with time of exposure to 0.34 ppm of SO_2, but the response of those grown in tailings sand was significantly greater ($p < 0.05$) for all species (Table 2). It appears that conifers grown in tailings sand do not have the same physiological resistance to SO_2 as those grown in native soils. This resistance does not appear to be related to stomatal response, since in this case S uptake was the same for both rooting media. The capability of these species to detoxify SO_2 appears to be reduced through either the presence of other toxic substances or the differences in the nutrient regime. Sensitivity of plants to atmospheric pollutants has been shown to be affected by the supply of essential nutrients (Guderian, 1977; Jager and Klein, 1976).

The time required for visible symptom development was the same whether the plants were grown in native soil or tailings sand (Table 2). This suggests that the use of visible symptoms for evaluating plant sensitivity to SO_2 under different environmental conditions may not be accurate.

CONCLUSIONS

Deciduous woody species were significantly more sensitive to fumigation with 0.34 ppm of SO_2 than were evergreen species in both the development of visible foliar symptoms and the reduction of NAR with time of fumigation. There were, however, no significant differences among species within the two groups.

Conifers grown in tailings sand were found to be substantially more sensitive to 0.34 ppm of SO_2 than were those grown in a Degraded Dystric Brunisol, based on the observation that the rate of reduction of NAR with fumigation was significantly faster in plants grown in tailings. Only *Pinus banksiana* showed a response to the tailings sand before fumigation with SO_2. Visible symptom development did not reflect the drop in the gas exchange rate with fumigation, and S uptake was similar in plants grown in native soil or tailings sand. It appears that the tailings sand resulted in the absorbed SO_2 being metabolically more damaging rather than increasing the total pollutant uptake. It is important to note, however, that if uptake of soil S from the Brunisol was greater than that from the tailings sand, gaseous S uptake by the tailings-grown conifers would be greater. No attempt was made to distinguish between soil and atmospheric S uptakes.

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LITERATURE CITED

1. Addison, P.A. 1980. Ecological bench-marking and biomonitoring for detection of airborne pollutant effects on vegetation and soils. Rep. 111. Alberta Oil Sands Environ. Res. Program, Edmonton, Alberta.

2. Addison, P.A., and J. Baker. 1979. Interim report on ecological benchmarking and biomonitoring for detection of airborne pollutant effects on vegetation and soils, 1975 to 1978. Rep. 46. Alberta Oil Sands Environ. Res. Program, Edmonton, Alberta.

3. Addison, P.A., and K.J. Puckett. 1980. Deposition of atmospheric pollutants as measured by lichen element content in the Athabasca Oil Sands area. Can. J. Bot. 58:2323-2334.

4. Barrie, L.A., and D.M. Whelpdale. 1978. Background air and precipitation chemistry. p. 124-259. *In* F. Fanaki (ed.) Meteorology and air quality field study in the AOSERP study area, March 1976. Rep. 27. Alberta Oil Sands Environ. Res. Program, Edmonton, Alberta.

5. Black,V.J., and M.H. Unsworth. 1979. Effects of low concentrations of sulphur dioxide on net photosynthesis and dark respiration of _Vicia faba_. J. Exp. Bot. 30:473-483.

6. Carson, J.A., J.M. Crepin, and P. Nemunis Siugzdines. 1972. A sulphate-sulphur method used to delineate the sulphur status of soils. Can. J. Soil Sci. 52:278-281.

7. Chan, C.C.Y. 1975. Determination of total sulphur in vegetation by a turbidimetric method following oxygen flask combustion. Anal. Lett. 8:655-663.

8. Dreisinger, B.R. 1965. Sulphur dioxide levels and the effects of the gas on vegetation near Sudbury, Ontario. Presented at the 58th Annual Meeting of the Air Pollution Control Association, Toronto, Ontario.

9. Furukawa, A., T. Natori, and T. Totsuka. 1980. The effect of SO_2 on net photosynthesis in sunflower leaf. p. 1-8. *In* Studies on the effects of air pollutants on plants and mechanisms of phytotoxicity. Res. Rep. 11. Institute of Environmental Studies. Yatabe, Japan.

10. Gates, D.M. 1966. Transpiration and energy exchange. Q. Rev. Biol. 41:353-364.

11. Guderian, R. 1977. Air pollution. Phytotoxicity of acidic gases and its significance in air pollution control. Ecological Studies 22. Springer-Verlag, Berlin.

12. Jacobson, J.S., and A.C. Hill. 1970. Recognition of air pollution injury to vegetation: a pictorial atlas. Air Pollution Control Association, Pittsburgh, Penn.

13. Jager, Von H.J., and H. Klein. 1976. Modellversuche zum Einfluss der Nahrstaffversorgung auf die SO_2-Empfindlichkeit von Planzen. Eur. J. For. Path. 6:347-354.

14. Keller, T. 1980. The effect of a continuous springtime fumigation with SO_2 on CO_2 uptake and structure of the annual ring in spruce.

Can. J. For. Res. 10:1-6.

15. Keller, T., and J. Bucher. 1976. Zur SO_2-Empfindlichkeit der Laubbaumarten. Schweiz. Z. fur Forstwes. 7:476-484.

16. Legge, A.H., D.R. Jacques, G.W. Harvey, H.R. Krouse, H.M. Brown, E.C. Rhodes, M. Nosal, H.U. Schellhase, J.M. Mayo, A.P. Hartgerink, P.F. Lester, R.G. Amundson, and R.B. Walker. 1978. Sulphur gas emissions in the boreal forest: the West Whitecourt case study. Whitecourt Environ. Study Group, Environ. Sci. Cent. Kananaskis, Alberta.

17. Majernik, O., and T.A. Mansfield. 1972. Stomatal responses to raised atmospheric CO_2 concentrations during exposures of plants to SO_2 pollution. Environ. Pollut. 3:17.

18. Malhotra, S.S., and R.A. Blauel. 1980. Diagnosis of air pollutant and natural stress symptoms on forest vegetation in western Canada. Environ. Can., Can. For. Serv., North. For. Res. Cent. Edmonton, Alberta. NOR-X-228.

19. Malhotra, S.S., and A.A. Khan. 1982. Biochemical and physiological impact of major pollutants. *In* M. Treshow (ed.) Atmospheric pollution and plant life. John Wiley and Sons Ltd., London (in press).

20. Miller, J.E., and P.B. Xerikos. 1979. Residence time of sulphite in SO_2 'sensitive' and 'tolerant' soybean cultivars. Environ. Pollut. 18:259-264.

21. Mudd, J.B. 1975. Sulphur dioxide. p. 9-22. *In* T.T. Kozlowski and J.B. Mudd (eds.) Responses of plants to air pollution. Academic

F-2036 (REV. 12/69) FD-6

Press Inc., New York.

22. O'Gara, P.J. 1922. Sulfur dioxide and fume problems and their solutions. Ind. Eng. Chem. 14:744.

23. Sestak, Z., P.G. Jarvis, and J. Catsky. 1971. Criteria for the selection of suitable methods. p. 1-48. *In* Z. Sestak, J. Catsky, and P.G. Jarvis (eds.) Plant photosynthetic production manual of methods. Dr. W. Junk, N.V., Publishers, The Hague.

24. Shelfentook, W. 1978. An inventory system for atmospheric emissions in the AOSERP study area. Rep. 29. Alberta Oil Sands Environ. Res. Program, Edmonton, Alberta.

Table 1--Net CO_2 assimilation rates (mean ± 95% confidence limits) of woody plants before fumigation with SO_2.

Species	Net assimilation rate Dystric Brunisol	 Tailings sand
	mg CO_2 g^{-1} h^{-1}	
Salix sp.	19.0 ± 11.7	
Populus tremuloides	17.7 ± 5.5	
Betula papyrifera	14.6 ± 2.8	
Alnus crispa	13.7 ± 2.2	
Ledum groenlandicum	7.6 ± 5.0	
Pinus banksiana	6.4 ± 1.5	2.9 ± 1.1
Picea glauca	5.4 ± 0.8	6.1 ± 3.2
Picea mariana	5.0 ± 1.5	6.1 ± 0.9

Table 2--Regression coefficients of the percentage change in net assimilation rate (NAR) with time, sulfur intake, and time required for visible symptom development with SO_2 for woody plants growing in native soil and in tailings. Sulfur uptake and visible symptom values are means ± 95% confidence limits.

Substrate	Species	Prefumigation slope	Fumigation slope†	Sulfur uptake	Visible symptom initiation‡
				— $\mu g\ g^{-1}\ d^{-1}$ —	— days —
Dystric Brunisol	*Salix* sp.	-1.1	-9.9*	440 ± 230	2 ± 1
	Betula papyrifera	+1.7	-8.7*	660 ± 240	5 ± 2
	Populus tremuloides	+2.1	-7.7*	260 ± 80	4 ± 1
	Alnus crispa	+0.2	-4.3*	480 ± 170	3 ± 1
	Ledum groenlandicum	-3.2	-2.2*	270 ± 170	9 ± 3
	Picea glauca	0	-2.1*	150 ± 60	21 ± 4
	P. mariana	+0.5	-1.7*	80 ± 30	15 ± 5
	Pinus banksiana	+1.5*	-1.3*	90 ± 40	11 ± 2
Tailings Sand	*Picea glauca*	+3.2	-5.2*	130 ± 20	15 ± 0
	P. mariana	-6.3	-5.0*	110 ± 70	15 ± 0
	Pinus banksiana	-3.2	-6.5*	70 ± 70	13 ± 10

† Vertical lines join slopes that are not significantly different ($p < 0.05$) by the Simultaneous Test Procedure.

‡ Means that are joined by a vertical line are not significantly different ($p < 0.05$) in a Student-Newman-Keuls test.

* Indicates that there is a significant ($p < 0.05$) relationship between NAR and time (i.e., slope is significantly different from zero).

Figure 1. The Alberta Oil Sands Environmental Research Program study area and plant collection sites. A indicates native soil site location and B indicates tailings sand site location.

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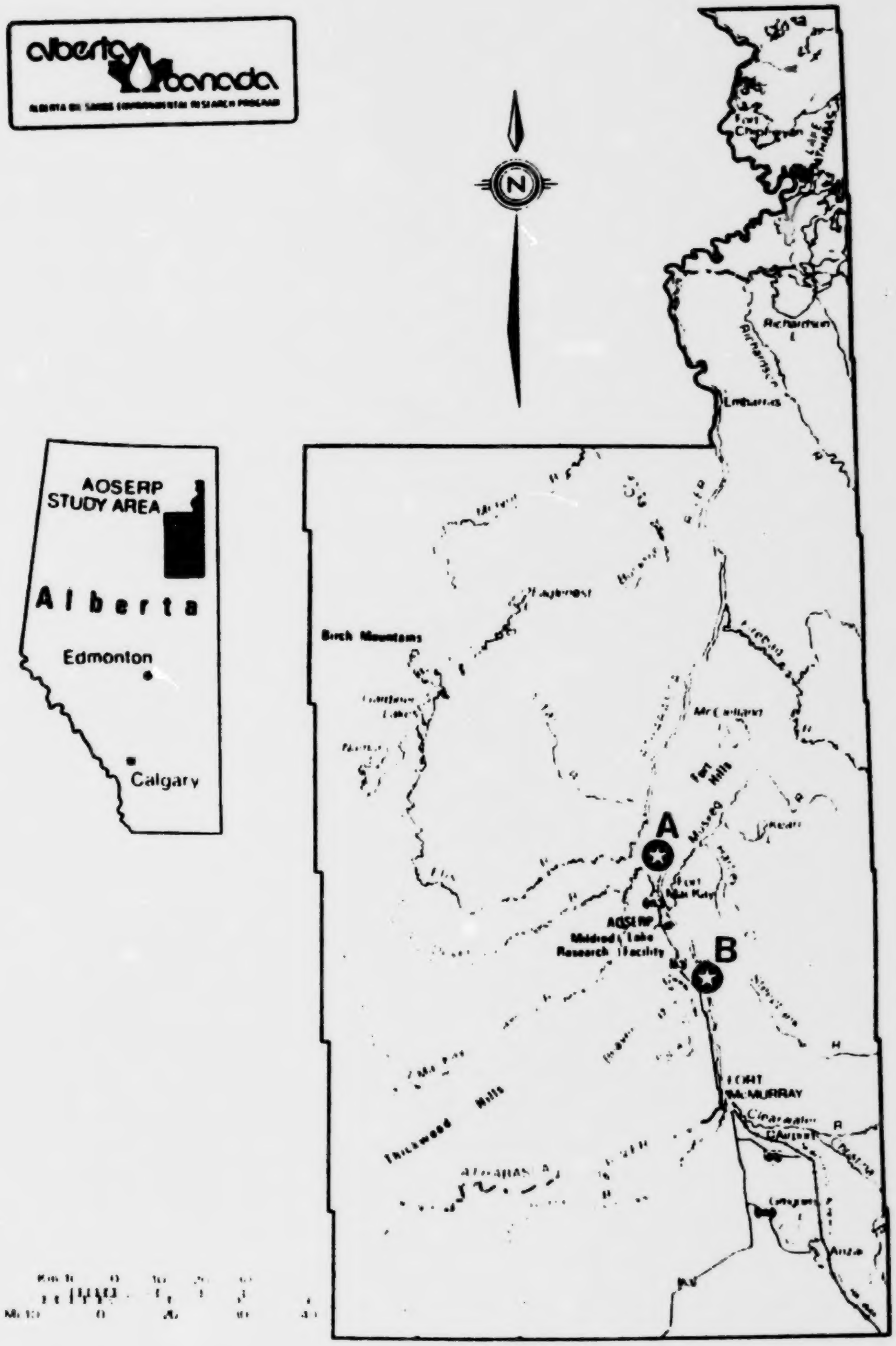

QUANTIFICATION OF BRANCH DWELLING LICHENS FOR THE DETECTION OF AIR POLLUTION IMPACT

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ABSTRACT

In the Athabasca Oil Sands area of Alberta, a study was carried out to test a lichen community transplant technique and to determine the influence of oil sands emissions on lichen cover.

Measurement errors in lichen cover may be great (up to 100%) particularly when the cover is small. These errors must be evaluated before the interpretation of response to pollutants is possible.

Transplantation of lichen communities from their natural environment may be detrimental but it is possible to define habitats that do not negatively influence lichen cover. White spruce and jack pine habitats appeared to be suitable for transplants even over a period of years.

Significant reductions in lichen cover (except *Hypogymnia*) occurred close to pollution sources and could not be accounted for by measurement error or transplantation effects. In spite of low annual SO_2 concentration in the area (0.003 ppm), lichen cover was detrimentally affected.

INTRODUCTION

Lichens are well-known for their sensitivity to air pollution and, in both Europe and North America, they have been used extensively to detect changes in air quality (James 1975). Many mapping studies have used the distribution of selected sensitive species to indicate the pattern of pollutant impingement (Gilbert 1969, Rose 1973). This has only been possible however, in areas where there was sufficient impact of the pollutants to eliminate certain lichen species from the immediate vicinity of the source.

In the Athabasca Oil Sands area of Alberta, there has been very little obvious damage to the forest vegetation that can be related to air pollution (Addison and Baker 1979). Although some lichen degradation has occurred close to the pollution source, no species had been eliminated from any site examined (Addison and Puckett 1980). There were, however, obvious changes in cover and luxuriance of the dominant lichen species.

The aim of this study was to develop a method to use the arboreal lichen community growing on branches as a measure of air pollution impact. Tree branch dwelling lichens are more exposed to air pollutants and hence, are less toxitolerant than either those on the ground or on tree trunks (Le Blanc and Rao 1975).

The oil sands area presented an ideal situation to test the method since both industry (Douglas and Skorepa 1976) and government (Addison 1980) were involved in long-term biomonitoring for air pollution effects. In addition, there was substantial air quality information that had been collected by the Alberta Oil Sands Environmental Research Program (Walmsley and Bagg 1977, Barrie 1978, Strosher 1979).

MATERIALS AND METHODS

Black spruce (*Picea mariana* (Mill.) BSP.) branches with lichen stands of mainly fruticose and foliose growth forms were selected from the Fort MacKay area about 25 km north of the Suncor Inc. oil extraction operation (Fig. 1).

The lichen species present were grouped into four classes (Table 1) based upon their morphology and ease of recognition. Quantification of the cover and frequency of the species groups was accomplished using a 15 X 20 cm gray-card quadrat and photographs. Each branch to be sampled was marked and labelled and the lichens were moistened slightly with an atomizer to make them pliable. Since black spruce branches are oriented mainly on a horizontal plane, postioning of the card was possible without damaging the lichen stand. Intervals (1 cm) were marked along one side of the quadrat so that the exact position of the branch was recorded in the photograph. Since almost all lichens grow on the top and sides of branches, the entire stand was included in a photograph taken from directly above the branch. Photographs were taken at a focal distance of about 45 cm with a 50 mm lens and Kodachrome 64 film (ASA 64, Eastman Kodak Co.). Care was taken to ensure that the camera was directly perpendicular to the quadrat card. Natural light and the relatively fast film speed instead of a flash resulted in better resolution of the species present and a far simpler system to operate in the field. Only diffuse light was used and direct sunlight was shielded from the quadrat by a 1 X 2 m screen on 4 m poles. The photographs were analyzed using a modified point quadrat technique similar to that used by Mathis (1974). Transparencies were projected onto a 45 X 60 cm rear-projection screen divided into 100 units. The number of units in which each lichen species occurred was recorded. Owing to the very large size of the units (3 cm^2), a substantial exaggeration of cover was expected using this technique (Goodall 1952). In order to reduce this error, the average percentage cover of each species group in a unit was estimated. Twelve quadrats whose lichen densities ranged from the lowest to the highest found in the field were examined. The percentage of each unit that each species group covered when it occurred was estimated. Actual percentage cover for each quadrat therefore, was determined by multiplying the number of units containing a species by the average cover per unit.

In 1976, a set of transplants collected from the Fort MacKay area was estimated at Fort MacKay to determine the influence of microenvironment on the lichen stands in a relatively pollution-free location. Duplicate branches were placed at a height of 1.5 m on the top of 4 X 4 cm posts in a clearing and under jack pine (Pinus banksiana Lamb.), white spruce (Picea glauca (Moench) Voss), aspen (Populus tremuloides Michx.) and black spruce. In addition to the transplants, 10 replicate naturally occurring lichen stands were selected in a black spruce community adjacent to the experimental transplant sites. All lichen stands were photographed each year from 1978 to 1980.

In 1977, a second set of transplants were established in the vicinity of the Suncor Inc. pollution source (Fig. 1). Five sites (A-E) were selected in a southwesterly direction at respective distances of 2.8, 3.4, 4.2, 5.3, and 8.3 km from the power house stack. The exact positions of the sites were dictated by their accessibility. Ten branches were transplanted into each site under 5 m white spruce trees at a height of 1.5 m. The transplants were photographed in 1977 when they wre installed and in 1978, 1979, and 1980.

RESULTS AND DISCUSSION

The dominant lichen species occurring on black spruce branches in the Athabasca Oil Sands area (Table 1) form a plant community that was similar to that on Scots pine (Pinus sylvestris L.) in Norway (Horntvedt 1975), on black spruce in northern Ontario (Yarranton 1972), and on lodgepole pine (Pinus contorta Loudon var. latifolia Engelm.) on the eastern slope of the Rocky Mountains (Skorepa and Vitt 1976). Douglas and Skorepa (1976) identified several other lichen species on black spruce branches in the Athabasca Oil Sands area that were not found during this study. Even though most species (Table 1) could be recognized in the photographs, the species were placed into four groups readily recognizable by a non-lichenologist.

MEASUREMENT ERRORS

Before any measurement can be used to quantify air pollution impact, assessment of the measurement errors must be made. This was accomplished through the use of naturally occurring lichen quadrats that were presumably in equilibrium with their environment outside of the SO_2 impingement area. There was no significant ($p < 0.05$) change in the cover of any lichen species groups with time at Fort MacKay (Table 2). The null hypothesis of no difference must be accepted therefore, and the variation in these repetative measurements was assumed to represent error in the measurement itelf. Obviously, there may be other factors involved in the generation of the variation but it was expected that by assuming that all variation was caused by measurement impression, an over-estimate of the measurement error would be obtained.

The potential measurement errors (Table 2) were very high ranging from 10% of the measured mean for Evernia to 100% for Bryoria. The error estimates, however, were not related to the means and there was no correlation ($p < 0.05$) between the error and the mean. The values presented in Table 2 therefore, represent the imprecision in the lichen cover throughout the range of lichen cover measurements. This is extremely important since at low lichen covers, the measurement error may exceed 100%. In this technique therefore, the lichens must be present in large amounts before reasonably precise (± 10%) measurements can be obtained.

EFFECT OF TRANSPLANTATION

One of the major criticisms of transplant studies is that the lichens are divorced from their natural habitat and often their condition worsens even in pollution-free areas (Kauppi 1976). The influence of a change in environment on lichens is not well understood (Farrar 1973) and measurements on the impact of transplanting lichens into differing environments has not been reported (Ferry and Coppins 1979). In order to determine the influence of the various habitats on the cover of each lichen species group, an analysis of variance was carried out on the yearly changes in cover between naturally occurring and each transplant habitat (Table 3). Since all measurements were taken outside of the impingement zone (Addison 1980), changes in the tranplants should parallel those in the naturally occurring lichens. All habitats except white spruce showed significant

($p < 0.05$) differences from naturally occurring lichens in the yearly change in cover of at least one lichen species (Table 3). The inability to demonstrate a difference in lichen cover change between the white spruce transplant and natural habitat does not appear to be a result of either inadequate sampling or differences in the variances of the two samples. We must conclude therefore, that the two habitats elicited the same response in the lichens and that it is possible to transplant lichens into a new environment without significant detrimental effects. It should be noted that the effect on lichen cover in the jack pine habitat was a significant ($p < 0.5$) decrease in the yearly change in cover of Cetraria. Since the amount of change was actually less, jack pine may be a suitable habitat for the long-term stability of these lichen quadrats.

EFFECTS OF AIR POLLUTANTS

The responses of the various lichen species in the vicinity of oil sands operations were variable and produced a complex pattern. Hypogymnia (H. physodes and Parmelia sulcata) was the only species group that did not show a significant ($p < 0.5$) reduction in cover with time at the closest site (A) to the pollutant source (Table 4). A number of lichen mapping and transplant studies, however, have shown substantial changes to both H. physodes (Skye 1968, Schonbeck 1974) and to P. sulcata (Le Blanc et al. 1971, Le Blanc and Rao 1973). The major reason for this apparent discrepancy appears to be the level of SO_2 in the areas of interest. The critical annual SO_2 concentration reported for these species were 0.03 to 0.05 ppm in England and Wales (Hawksworth and Rose 1970, Tallis 1974) and 0.04 ppm in the Sudbury area (Le Blanc and Rao 1973). Since the annual SO_2 concentration at the closest air monitoring station (adjacent to site B) was 0.003 ppm (Strosher 1979), it is not surprising that no measureable response in the Hypogymnia group was observed.

There was a significant ($p < 0.001$) reduction in both Bryoria fuscescens and the Cetraria group (C. pinastri and C. halei) at all of the sites within 8.3 km from Suncor operations. B. fuscescens showed a rapid and consistent decrease in cover from year to year at sites B, C, and D but not at sites A and E (Table 4). At site A, the response of cover appeared to be greater than in B, C, and D and there was a virtual elimination (within measurement error) of this species after only 3 years. *Bryoria* has been shown to be particularly sensitive to lower levels of SO_2 (Skorepa and Vitt 1976). At site E on the other hand, a significant ($p < 0.05$) reduction in the cover *B. fuscescens* only occurred in 1980. This reduction in cover after severel years with no change may be a response to an increase in SO_2 concentration possibly owing to the startup of a second oil extraction plant (Syncrude). Air quality measurements adjacent to site B indicated an increase in annual SO_2 concentration from 0.0027 ppm in 1977 (Strosher 1979) to ppm in 1980 (Air Quality Division, Alberta Environment records).

The *Cetraria* group showed significant ($p < 0.001$) reductions in cover with time at all sites but at sites D and E the reduction was only in 1980. At sites A, B, and C, reduction in cover was only apparent after two years of exposure (Table 4). The longer time period required for a significant response of *Cetraria* to pollutants as compared with *Bryoria* appears to reflect the known differences in sensitivity between these two species (Case 1970). A large portion of that difference can be accounted for by the foliose versus fruticose growth form (Gilbert 1970). It was not possible to account for the increase in the cover of *Cetraria* at site B in 1978 (Table 4) but the change was too great to be interpreted as caused by either measurement error or a transplantation effect or a combination of the two.

Evernia (*Evernia mesomorpha*, *Usnea* spp., and *Ramalina* spp.) showed the greatest site to site variability of all species groups. There were significant ($p < 0.001$) reductions in cover of this species group at sites A, C, and D Table 4) but, surprisingly, not at site B. The lack of response of *Evernia* at site B could not be accounted for by either pollutant deposition pattern (Barrie 1978, Strosher 1979) or the concentration of pollutants in lichen tissue (Addison and Puckett 1980). In addition, both *Bryoria* and *Cetraria* groups showed a significant ($p < 0.001$) change in cover at site B. No hypothesis is offered for the lack of response of Evernia to air pollutants at site B. Since site E showed no significant ($p < 0.05$) reduction in Evernia cover with time, it appears that this species group is less sensitive than either Bryoria or Cetraria. The discrepancies in the Evernia data set however, makes it impossible to make a definitive statement.

In general, lichen cover estimates in the vicinity of oil sands operations indicated a significant response that correlated with the air pollution pattern in the area. Site A, B, and C could not be distinguished from each other based on the change in lichen cover with time and it appears that where the summer sulphation rate is about 0.06 mg SO_3 100 cm^{-2} d^{-1} in the lichen microenvironment (Table 4), there is a significant reduction in the cover of the dominant arboreal macrolichen species (excluding Hypogymnia physodes and Parmelia sulcata). Site D had significant reductions in lichen cover also but, since the magnitude of the reduction in Evernia cover was less and Cetraria took 3 years to change, this site was considered less affected than A, B, or C. Site E, although not a true control, responded only slightly and showed reductions in the cover of Bryoria and Cetraria only in 1980.

LITERATURE CITED

Addison, P.A. 1980. Ecological bench-marking and biomonitoring for detection of airborne pollutant effects on vegetation and soils. Alberta Oil Sands Environmental Research Program Report No. 111. 48 pp.

Addison, P.A. 1982. Biomonitoring in the Athabasca oil sands area of Alberta: progress and pitfalls. Pages 331-367 in Symposium Workshop Proceedings: Acid Forming Emissions in Alberta and Their Ecological Effects. Alberta Department of Environment, Canadian Petroleum Association, Oil Sands Environmental Study Group, March, Edmonton. 648 pp.

Addison, P.A. and J. Baker. 1979. Interim report on ecological bench-marking and biomonitoring for detection of air-borne pollutant effects on vegetation and soils 1975 to 1978. Alberta Oil Sands Environmental Research Project Report No. 46. 41 pp.

Addison, P.A. and K.J. Puckett. 1980. Deposition of atmospheric pollutants as measured by lichen element content in the Athabasca Oil Sands area. Can. J. Bot. 58:2323-2334.

Barrie, L.A. 1978. The concentration and deposition of sulphur compounds and metals around the GCOS oil extraction plant during June 1977. Alberta Oil Sands Environmental Research Project Report No. 68. 248 pp.

Case, J.W. 1980. The influence of three sour gas processing plants on the ecological distribution of epiphytic lichens in the vicinity of Fox Creek and Whitecourt, Alberta, Canada. Water, Air, and Soil Pollut. 14:45-68.

Douglas, G.W. and A.C. Skorepa. 1976. Monitoring air quality with lichen: a feasibility study. Syncrude Canada Ltd. Environmental Research Monograph 1976. 269 pp.

Farrar, J.R. 1973. Lichen physiology: progress and pitfalls. Pages 238-282 in Ferry, B.W., M.S. Baddeley, and D.L. Hawksworth (eds.). Air Pollution and Lichens. Univ. of Toronto Press, Toronto. 389 pp.

Ferry, B.W. and B.J. Coppins. 1979. Lichen transplant experiments and air pollution studies. Lichenologist 11:63-73.

Gilbert, O.L. 1969. The effect of SO_2 on lichens and bryophytes around Newcastle upon Tyne. Pages 223-235 in Air Pollution, Proceedings of the First European Congress on the Influence of Air Pollution on Plants and Animals. Wageningen: Centre for Agricultural Publishing and Documentation.

Gilbert, O.L. 1970. Further studies on the effect of sulphur dioxide on lichens and bryophytes. New Phytol. 69:605-627.

Goodall, D.W. 1952. Some consideration in the use of point quadrats for the analysis of vegetation. Aust. J. Sci. Res. 5:1-41.

Hawksworth, D.L. and F. Rose. 1970. Qualitative scale for estimating sulphur dioxide air pollution in England and Wales using epiphytic lichens. Nature, Lond. 227:145-148.

Horntvedt, R. 1975. Epiphytic macrolichens or Scots pine related to air pollution from industry in Odda, Western Norway. Meddelelser fr Norsk. Institutt for Skogforskning 31:584-604.

James, P.W. 1975. Introduction. Pages 1-5 in Ferry, W.B., M.S. Baddeley, and D.L. Hawksworth (eds.). Air Pollution and Lichens. Univ. of Toronto Press, Toronto. 389 pp.

Kauppi, M. 1976. Fruticose lichen transplant technique for air pollution experiments. Flora, Bd. 165:407-414.

Le Blanc, F. and D.N. Rao. 1973. Effects of sulphur dioxide on lichen and moss transplants. Ecology 54:612-617.

Le Blanc, F. and D.N. Rao. 1975. Effects of air pollutants on lichens and bryophytes. Pages 237-272 in Mudd, J.B. and T.T. Kozlowski (eds.). Responses of Plants to Air Pollution. Academic Press, New York. 383 pp.

Le Blanc, F., G. Comeau, and D.N. Rao. 1971. Fluoride injury symptoms in epiphytic lichens and mosses. Can. J. Bot. 49:1691-1698.

Mathis, D.M. 1974. The effect of sulphur dioxide on lichens. Am. Biol. Teacher. October :418-420.

Rose, F. 1973. Detailed mapping in southeast England. Pages 77-88 in Gerry, B.W., M.S. Baddeley, and D.L. Hawksworth (eds.). Air Pollution and Lichens. Univ. of Toronto Press, Toronto. 389 pp.

Schonbeck, H. 1974. Eine methode zur erfassung der biolozischen wirkung von luftverunreinigung durch transplantierte flechten. Staub Reinhalt Luft 29:14-18.

Skorepa, A.C. and D.H. Vitt. 1976. A quantitative study of epiphytic lichen vegetation in relation to SO_2 pollution in western Alberta. Canadian Forestry Service, Northern Forest Research Centre, Edmonton. Inf. Rep. NOR-X-161. 26 pp.

Skyes, E. 1968. Lichens and air pollution: a study of cryptogamic epiphytes and environment in the Stockholm region. Acta Phytogeogr. Suec. 52:1-123.

Strosher, M. 1978. Ambient air quality in AOSERP study area, 1977. Alberta Oil Sands Environmental Research Program Report No. 30. 74 pp.

Tallis, J.H. 1964. Lichens and atmospheric pollution. Advmt. Sci. Lond. 21:250-252.

Walmsley, J.L. and D.L. Bagg. 1977. Calculations of annual averaged sulphur dioxide concentrations at ground level in the AOSERP study area. Alberta Oil Sands Environmental Research Report No. 19. 40 pp.

Yarranton, G.A. 1972. Distribution and succession of epiphytic lichens on black spruce near Cochrane, Ontario. Bryologist 75:462-480.

Table 1. Predominate macrolichens occurring on black spruce branches in the Athabasca Oil Sands area of Alberta. Group name indicates grouping used in transplant studies.

Species	Group
Evernia mesomorpha Nyl.	*Evernia*
Usnea spp. (tufted)	*Evernia*
Usnea spp. (pendulate)	*Evernia*
Ramalina spp.	*Evernia*
Hypogymnia physodes (L.) W. Wats.	*Hypogymnia*
Parmelia sulcata Tayl.	*Hypogymnia*
Cetraria halei W. Culb and C. Culb	*Cetraria*
C. pinastri (Scop.) S. Gray	*Cetraria*
Bryoria fuscenscens (Gyeln.) Brodo and D. Hawksw.	*Bryoria*

Table 2. Cover and measurement error estimates for naturally occurring lichen quadrats at Fort MacKay. Value are means ±95% c.l.

Sampling time	Lichen cover (%)			
	Evernia	Bryoria	Hypogymnia	Cetraria
1976	37.7 ± 5.2	0.36 ± 0.27	5.58 ± 0.76	0.53 ± 0.28
1978	34.4 ± 3.9	0.24 ± 0.18	6.58 ± 1.07	1.24 ± 0.64
1979	34.5 ± 5.1	0.15 ± 0.11	6.44 ± 0.91	1.09 ± 0.57
1980	36.6 ± 3.7	0.12 ± 0.08	5.69 ± 0.63	0.64 ± 0.44
Yearly variation between measurements	3.64 ± 1.14	0.21 ± 0.06	1.21 ± 0.31	0.67 ± 0.23

Table 3. Mean ±95% confidence limits of the yearly change in cover transplanted and naturally occurring lichen quadrats at Fort MacKay. Measurements were taken yearly over a five year period.

Habitat	Change in % lichen cover			
	Evernia	Bryoria	Hypogymnia	Cetraria
Naturally occurring black spruce	3.64 ± 1.14	0.21 ± 0.06	1.21 ± 0.31	0.67 ± 0.23
Transplants				
Jack pine	2.00 ± 1.04	0.25 ± 0.16	0.83 ± 0.53	0.34 ± 0.19[a]
Open	4.19 ± 3.75	0.56 ± 0.41[a]	1.55 ± 1.01	1.42 ± 1.00[a]
Aspen	8.93 ± 11.52[a]	0.38 ± 0.30	2.03 ± 2.08	0.57 ± 0.79
Black spruce	2.96 ± 2.13	0.56 ± 0.37[a]	1.21 ± 0.60	0.74 ± 0.48
White spruce	3.69 ± 1.62	0.24 ± 0.15	1.81 ± 1.14	0.91 ± 0.45

[a] Means are significantly ($p < 0.05$) different from the naturally occurring population of the same species group.

Table 4. Mean ±95% confidence limits of lichen cover at sites close to oil sands extraction plants.

Site	Distance from Suncor (km)	Sulphation rate[a] mg SO_3 100 cm^{-2} d^{-1}	Sample time	% lichen cover			
				Evernia	Bryoria	Hypogymnia	Cetraria
A	2.8	0.055	1977	33.6 ± 5.4[b]	1.2 ± 0.5[b]	10.1 ± 1.4	4.2 ± 1.1[b]
			1978	30.3 ± 4.9	0.6 ± 0.4	9.8 ± 1.3	3.7 ± 1.0
			1979	19.6 ± 9.0	0.1 ± 0.1	9.1 ± 1.2	1.7 ± 1.0
			1980	11.9 ± 7.4	0.1 ± 0.1	9.1 ± 0.7	0.3 ± 0.2
B	3.5	0.058	1977	37.0 ± 5.5	1.3 ± 0.2[b]	9.8 ± 1.4	2.9 ± 0.7[b]
			1978	29.8 ± 6.9	0.8 ± 0.4	9.4 ± 0.9	4.0 ± 0.5
			1979	29.9 ± 8.3	0.4 ± 0.2	9.4 ± 1.4	2.5 ± 0.8
			1980	24.3 ± 8.4	0.1 ± 0.1	10.2 ± 1.6	0.4 ± 0.2
C	4.2	0.060	1977	35.8 ± 3.9[b]	0.8 ± 0.2[b]	9.4 ± 1.6	4.0 ± 0.9[b]
			1978	19.1 ± 9.7	0.6 ± 0.3	10.0 ± 1.4	4.1 ± 0.8
			1979	9.7 ± 10.9	0.2 ± 0.2	9.6 ± 1.0	2.4 ± 0.7
			1980	9.5 ± 9.2	0	9.2 ± 0.9	0.4 ± 0.4
D	5.3	0.045	1977	32.4 ± 5.9[b]	0.7 ± 0.4[b]	8.2 ± 1.4	3.3 ± 0.8[b]
			1978	31.3 ± 8.2	0.6 ± 0.4	8.6 ± 1.1	3.9 ± 0.7
			1979	23.1 ± 10.3	0.2 ± 0.1	8.4 ± 1.6	3.2 ± 0.9
			1980	14.4 ± 10.1	0	8.5 ± 1.4	0.6 ± 0.4
E	8.3	0.035	1977	31.4 ± 5.0	0.8 ± 0.3[b]	8.4 ± 1.4	3.4 ± 1.0[b]
			1978	33.1 ± 4.0	1.1 ± 0.4	9.5 ± 1.3	3.6 ± 1.0
			1979	35.2 ± 3.4	1.0 ± 0.3	9.3 ± 1.2	3.5 ± 0.7
			1980	29.0 ± 4.9	0.3 ± 0.1	9.1 ± 1.7	1.5 ± 0.5

[a] Values are 1979 summer averages from within a white spruce canopy (Addison 1982).

[b] Indicates that there is a significant ($p < 0.001$) reduction in lichen cover with time. Others are not significant at 0.05.

PUBLICATIONS IN PREPARATION

TITLE: Influence of SO_2 concentration and the duration of exposure on ^{14}C incorporation in *Evernia mesomorpha.*

METHODS: Eight replicate samples were placed in each of a control and a fumigation cuvette. Gas concentrations of 0.1, 0.2, 0.4, 0.8 and 1.2 ppm SO_2 were maintained and monitored for periods of 1, 2, 4 and 8h (the latter time being the longest period for which the lichens remained fully hydrated). After the designated time, each sample was removed and divided into two portions for the measurement of ^{14}C incorporation and sulphur content.

RESULTS: *Evernia mesomorpha* responded to SO_2 at lower concentrations and duration of exposure than reported elsewhere. The form of the interaction between concentration and exposure duration has been described for both sulphur content and ^{14}C incorporation.

STATUS: All data are in hand. An article destined for the Journal of Environmental Quality will be prepared in the summer of 1983 and should be submitted in the fall.

FUTURE: No further work is planned in this research area in 1983.

TITLE: Distribution of SO_2 in forest canopies as measured by sulphation plates.

METHODS: Six replicate Huey sulphation plates were maintained at six heights in three forest types for a period of 8 months. Plates were placed 3m above the canopy, at canopy height (approx. 11m) and at heights of 6,3, 1.5 and 0.6m in aspen, jack pine and black spruce stands. Plates were exchanged about every 60d, extracted for SO_4 and analysed for S by Inductively coupled Argon Plasma - Atomic Emission Spectrometry (ICAP-AES).

RESULTS: The vertical distribution of sulphur deposition was highly dependent upon the type of forest stand. A second order polynomial was fitted to sulphation rate versus height for both pine and spruce stands. The amount of S deposition at 1.5m in the pine stand was 40-45% of the above canopy value whereas in the spruce stand it was only 13-19%. The aspen stand showed a totally different pattern with deposition increasing with height from the ground surface, then decreasing with height into the tree canopy and then increasing above the canopy. This appears to indicate penetration of the plume into the canopy.

STATUS: Only part of the collected data has been examined in detail. These data will be combined with data from Rocky Mountain House and with other Huey plate measurements (1977 - 78) from the oil sands area in a short paper on the pattern of S deposition in coniferous forests.

FUTURE: No further work is planned in this research area in 1983.

RESEARCH PLANNED
AND IN
PROGRESS

TITLE: The effect of SO_2 fumigation on physiology and biochemistry of jack pine seedlings.

OBJECTIVE: To measure physiological (water relations) and biochemical (sugar content, sulfur uptake) responses of jack pine seedlings to SO_2 fumigation at various concentrations for various durations, with emphasis on recording responses before visual symptoms appear.

METHODS: Part A: Jack pine seeds were planted in washed sand in four inch plastic pots and grown in a greenhouse until seedlings were ten weeks old. After four days of acclimation in the growth chamber, control or SO_2 fumigation runs were started at 0900. Randomly selected plants were removed after specified time intervals (0,2,6,10,24,48, 96 and 192 hours) for measurement of leaf resistance with a porometer, xylem tension with a Schclander bomb, fructose content, and sulfur content. The SO_2 concentrations used were 0, 0.1, 0.4, 0.6 and 1 ppm. Part B: Jack pine seeds were planted in washed sand in Ray Leach cone-tainers and grown in a growth chamber until seedlings were nine weeks old. After two days of acclimation for each set of plants, sampling runs were done in pairs. Controls were sampled on Monday to Wednesday and fumigated plants from Wednesday to Friday, at 0, 2, 6, 10, 24 and 47 hours. Randomly selected plants in each sample were ground in liquid nitrogen and oven dried in preparation for sugar assays. The SO_2 concentrations used are 0, 0.1, 0.2, 0.4, 0.8 and 1.0 ppm.

RESULTS: Part A: Final statistical analyses have not yet been completed, but the following tentative trends were observed. Leaf resistance was converted to leaf conductance for ease in handling data. Results indicate that leaf conductance decreased within two hours of the start of fumigation, and continued to decrease as duration of fumigation increased. This response was more dramatic at higher SO_2 concentrations. The data show considerable variation (large standard errors), so it is not yet known whether these results will be significant. Xylem tension also decreased as duration of fumigation increased, but in this case the response was not apparent until the plants were already visibly injured. It appears that, as expected, xylem tension decreased as a result of removal of the driving force of transpiration. Fructose content showed small but consistent decreases which began soon after fumigation and continued as duration increased. As with leaf conductance, these decreases were more dramatic at higher SO_2 concentrations. Sulfur content has not yet been determined.

Part B: This experiment is currently in progress and no results are available yet.

STATUS: Part A: Data are being prepared for multivariate statistical analyses which will indicate the relative importance of the variables measured and the significance of the observed trends. Sulfur content is being determined by the oxygen flask combustion technique followed by ICP analysis, and data should be available soon. Once all data are in hand and analyzed statistically, results will be synthesized and interpreted.

Part B: The first pair of concentrations (0 and 1.0 ppm) has been done. There are four more pairs to run, and provided that all equipment functions adequately, fumigation should be completed by the end of March. Following this, biochemical assays of sugar content will be performed.

FUTURE: An article describing the effects of SO_2 fumigation on physiology and biochemistry of jack pine seedlings, based on results from Parts A and B, will be prepared and submitted for review.

TITLE: Variability of soil chemical properties in contaminated coniferous forest soils.

OBJECTIVE: To quantify the soil chemical variability in contaminated soils in the Athabasca Oil Sands area.

METHODS: A 25 m^2 plot 2.8 km south of Suncor Inc. oil extraction plant was established and 25 samples from each of the 4 surface horizons (LFH, Ae, Bm and BC) were collected. Sampling locations were at 1m intervals. Samples were frozen prior to analysis. A subsample (2 - 5g) was removed, air-dried, ground to pass a 100 mesh screen and is currently being digested by HNO_3 - $HClO_3$ HF - HCl. Total element content of S, P, Ca, Mg, K, Na, Mn, Fe, Al, V and N, will be determined by ICAP - AES analysis. Total N and pH also will be determined. The remaining portion of each sample will remain frozen and will be extracted by various extractants, moist. Analysis will be carried out by ICAP - AES. The extractant(s) to be used have not been determined as some preliminary research is required to determine the most suitable extractants.

STATUS: Analysis for total element content should be completed by March 1983 whereas extraction and analysis of extractable element content will be completed during the summer.

FUTURE: These data will enable the interpretation of data collected from proposed lysimeter and leaching studies.

These data in conjunction with similar information from the Rocky Mountain House area will provide information on the variability of various soil chemical properties in soils under coniferous forests.

Changes in the chemical properties of soils owing to the addition of pollutants to soils.

OBJECTIVES:

(i) To determine the possible changes and the magnitude of any changes in soil chemical properties that could occur owing to artificial pollutants addition.

(ii) To determine if any changes in the soil chemical properties have occurred or are occurring under matured conditions owing to pollutant additions.

METHODS:

(i) A lab study will be carried out using reconstituted soil columns and leaching with known amounts of acids and/or metals. Total and extractable elemental concentrations of the various soil horizons will be determined. The elemental content of the leachates will also be determined.

(ii) A field study will be done using zero tension lysimeters just below the soil surface at the Ae - Bm interface in a contaminated and an uncontaminated site. The leachate will be analyzed for various elemental content. In addition sequential soil sampling will be taken at given intervals to monitor any possible changes in soil chemical properties.

STATUS: The details of the field and lab studies are being worked out. Both studies will be started this year.

FUTURE: Extrapolation of lab results to the field situation is difficult. However, by comparing what could occur in the worst possible case and what is actually happening in the field it is hoped that potential changes that could occur in the soil may be predicted. Whether the predicted changes could significantly affect the vegetation would require further study.

FUTURE PLANS

FUTURE PLANS

1983 GOALS

1. Submit an article on the influence of SO_2 concentration and duration of exposure on the ^{14}C incorporation by *Evernia mesomorpha*.
2. Prepare a short publication on the distribution of SO_2 in forest canopies.
3. Continue to study the influence of SO_2 concentration and duration of exposure on jack pine metabolism.
4. Determine the variation in soil chemistry in contaminated forest soils.
5. Submit a journal article on the effect of SO_2 on superoxide dismutase activity in jack pine.
6. Submit an article on plant metabolic changes caused by soil contamination.

POST-1983 GOALS

In the oil sands area, there appear to be two potential impacts of air pollution.

1. Significant changes in the chemistry of soils and subsequent impact on native plant species.
2. Direct impact by SO_2 mediated by other pollutants or environmental conditions.

In order to address these potential impacts and to accomplish our general objectives listed in the introduction, two convergent projects are anticipated: one initially dealing with soil chemical changes as a result of pollutant deposition and the other starting with plant physiological responses to single pollutants. As these projects merge, the influence of factors such as soil nutrition, pollutant gas mixtures, soil contamination, plant age and the physical environment on the growth and survival of native plant species can be evaluated. With sufficient understanding of the processes involved in pollutant impact, a predictive capability may be achieved.

POOR

QUALITY

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